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Polymorphism in a lincRNA Associates with a Doubled Risk of Pneumococcal Bacteremia in Kenyan Children

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Bacteremia (bacterial bloodstream infection) is a major cause of illness and death in sub-Saharan Africa but little is known about the role of human genetics in susceptibility. We conducted a genome-wide association study of bacteremia susceptibility in more than 5,000 Kenyan children as part of the Wellcome Trust Case Control Consortium 2 (WTCCC2). Both the blood-culture-proven bacteremia case subjects and healthy infants as controls were recruited from Kilifi, on the east coast of Kenya. *Streptococcus pneumoniae* is the most common cause of bacteremia in Kilifi and was thus the focus of this study. We identified an association between polymorphisms in a long intergenic non-coding RNA (lincRNA) gene (AC011288.2) and pneumococcal bacteremia and replicated the results in the same population (p combined = 1.69×10^{-9} ; OR = 2.47, 95% CI = 1.84–3.31). The susceptibility allele is African specific, derived rather than ancestral, and occurs at low frequency (2.7% in control subjects and 6.4% in case subjects). Our further studies showed AC011288.2 expression only in neutrophils, a cell type that is known to play a major role in pneumococcal clearance. Identification of this novel association will further focus research on the role of lincRNAs in human infectious disease.

Introduction

Bacteremia is a common pathway in the progression to death from severe pneumonia, meningitis, and sepsis,

which together account for an estimated 3 million deaths each year globally in children under the age of 5 years. Even in developed countries the mortality rate from bacteremia remains unacceptably high.^{1–3} The leading bacterial

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cause of death in young children worldwide is *Streptococcus pneumoniae* (pneumococcus), and 14.5 million episodes of serious pneumococcal disease occur in young children annually.⁴ A key question is why only a proportion of individuals develop invasive disease despite widespread exposure and asymptomatic carriage of bacteria. Host genetic factors play an important role in explaining inter-individual variation in susceptibility to different infectious diseases.⁵ However, the relevant genes for bacteremia susceptibility remain largely unknown.

To identify genetic correlates of bacteremia susceptibility, we conducted a genome-wide association study (GWAS) in Kenyan children, a population with a major disease burden,⁶ as a part of the Wellcome Trust Case Control Consortium 2 (WTCCC2). Bacteremia is a heterogeneous phenotype and immune responses and genetic variants affecting susceptibility are likely to be at least partially pathogen specific. We therefore focused on bacteremia caused by *S. pneumoniae*, the most common bacteria found in our study. In addition, all-cause bacteremia was analyzed in order to assess the possible role of genetic risk factors for bacteremia regardless of its etiology.

Subjects and Methods

Study Design

To identify host genetic determinants of susceptibility to invasive pneumococcal disease in African children, we performed a two-stage GWAS of pneumococcal bacteremia in 542 Kenyan children with culture-confirmed disease and 4,013 healthy control subjects. 429 case subjects and 2,677 control subjects were included in the discovery phase analysis, with 113 case subjects and 1,336 control subjects included in the replication analysis. To identify determinants of invasive bacterial disease irrespective of the pathogen, we further performed a GWAS of culture-confirmed all-cause bacteremia in the same population of Kenyan children (discovery phase, 1,536 case subjects; replication phase, 434 case subjects). Adopting a Bayesian framework, we considered evidence for shared effects at loci associated with pneumococcal disease and all-cause bacteremia, across pathogens commonly causing bacteremia in this population. Finally, we characterized disease-associated genetic variation identified in the study, analyzing tissue-specific expression of implicated transcripts in immune cell subsets. A detailed study workflow is described in Figure S1.

Study Participants

All study participants were residents of Kilifi District on the coast of Kenya. Case subjects were recruited among children younger than 13 years of age who were admitted to Kilifi District Hospital (KDH) in Kenya between 1st August 1998 and 30th October 2010. Blood cultures were investigated from everyone admitted (unless they were admitted for elective procedures or because of minor accidents) using the BACTEC 9050 system. Children with bacteria present in their bloodstream were defined as case subjects (*Corynebacterium* bacteria, *Bacillus* species, coagulase-negative *Staphylococcus*, *Staphylococcus saprophyticus*, and Viridans group *Streptococcus* were excluded as contaminants). The annual incidence of bacteremia in Kilifi between August 1998 and July 2002 was estimated to be 505

cases per 100,000 children who were less than 5 years of age,⁶ but the incidence has since decreased.⁷

Control subjects were selected from children born consecutively within the same Kilifi region between 1st May 2006 and 30th April 2008 and represent the case subjects closely in terms of sex, ethnic group, and geographic area of residence. Although the control individuals are part of a birth cohort study and thus aged less than 12 months at the time of recruitment to the study, we have been able to review their follow-up data in terms of development of bacteremia ($n = 12$), mortality ($n = 49$), etc. (See further demographic details of case and control subjects in Table S2.) Table S3 shows the distribution of the most common bacterial isolates identified from bacteremia case subjects in the discovery and replication sets. The final discovery set included 1,536 blood-culture-proven bacteremia case subjects (of whom 429 were pneumococcal) and 2,677 healthy infants as control subjects. Individuals in the replication set were enrolled during the end of the collection period and included 434 bacteremia case subjects (of whom 113 were pneumococcal) and 1,336 control subjects.

Ethical approval was granted by the Kenya Medical Research Institute (KEMRI) National Scientific Steering and Research Committees and the Oxford Tropical Research Ethics Committee (OXTREC). Informed consent was obtained from all subjects.

DNA Sample Preparation

Genomic DNA was extracted at the Kenya Medical Research Institute (KEMRI)-Wellcome Trust Collaborative Programme in Kenya, using the QIAamp DNA blood mini kit (QIAGEN) and shipped to the Wellcome Trust Centre for Human Genetics, University of Oxford, for further processing. Genomic DNA was whole-genome amplified at the GeneService laboratory with GenomiPhi (GE HealthCare) scaled to amplify 40–50 μ g of DNA. Quality of the whole-genome amplified DNA was assessed at the Wellcome Trust Sanger Institute as described elsewhere⁸ before genotyping.

Genome-wide Genotyping and Quality Control

Whole-genome amplified samples from case and control subjects were genotyped on the genome-wide Affymetrix SNP 6.0 chip at the Affymetrix service laboratory. Genotypes were called with a modified version of the Chiamo software⁹ for all samples passing the Affymetrix laboratory quality control measures. Sample QC was performed as described elsewhere⁸ and details are provided in Table S1 and Figures S1–S3. Analysis of pairwise allele sharing identified 68 duplicate pairs and 6 triplicates (Figure S4). Phenotypic information suggests that the majority of these duplicate and triplicate individuals were unintentionally recruited to the study in Kenya more than once rather than being sample handling problems; therefore, one of each of the duplicate pairs or triplicates was included in the analysis (a case rather than a control subject was included in the analysis; otherwise the sample with a higher call rate was included). First-degree relatives (genome-wide IBD sharing probability > 0.4 ; 117 individuals) were removed from the main analysis. The following criteria were used to exclude 102,896 unreliable SNPs: minor allele frequency (MAF) $< 1\%$ (50,322 SNPs), $\text{info} < 0.975$ (53,419 SNPs), Hardy-Weinberg equilibrium $p < 1 \times 10^{-20}$ (18,288 SNPs), plate effect $p < 1 \times 10^{-6}$ (7,382 SNPs), and SNP missingness $> 2\%$ (34,430 SNPs). Genotyping cluster plots of each SNP with $p < 1 \times 10^{-3}$ were visually inspected using Evoker,¹⁰ and SNPs with poor cluster separation were removed. After sample and SNP QC, 1,536 case subjects and 2,677 control subjects were

analyzed at 787,861 genotyped autosomal SNPs. Three main ethnicities—namely Chonyi, Giriama, and Kauma—were discernible with principal-components analysis (PCA) of the genome-wide data (Figure S5).

ImmunoChip Genotyping

Approximately 2,000 SNPs out of the total 200,000 SNPs were selected to be included in the ImmunoChip array¹¹ based on the initial association results of the bacteremia analyses. The replication set was genotyped with this array at the Wellcome Trust Sanger Institute. All the samples went through a similar QC process as described above for the discovery samples (Table S1) and 434 case subjects and 1,336 control subjects passed the QC. After excluding SNPs based on minor allele frequency < 1%, SNP call rate < 95% (<99% if MAF < 5%), and Hardy-Weinberg equilibrium $p < 1 \times 10^{-10}$, 143,100 SNPs remained for the further analyses. The same ethnicities were detectable by PCA in the replication sample set as in the discovery analysis (Figure S6). As the ImmunoChip genotyping was performed before the imputation, these genotypes were mainly utilized to account for population stratification and relatedness in the later replication analyses.

Imputation and Association Analyses

We performed whole-genome imputation using the 1000 Genomes Phase I data as a reference panel. Genotypes were pre-phased using SHAPEIT¹² before imputation with IMPUTE2.¹³ Only samples and SNPs passing the QC were included for pre-phasing and imputation. SNPs with potentially unreliable imputation were filtered out based on MAF (<2%), imputation info value (<0.8), and Hardy-Weinberg equilibrium ($p < 1 \times 10^{-10}$). 10,996,499 imputed autosomal SNPs that passed the QC were analyzed for additive and genotypic models using SNPTEST2,¹⁴ taking the imputed genotype uncertainty (frequentist score test) and the first two principal components (PCs) of genetic structure into account. The genomic control parameter λ for bacteremia overall and pneumococcal bacteremia after imputation and QC were 1.043 and 1.013, respectively (see the QQ plots in Figure S7). At associated SNPs, statistical tests were also performed using a linear mixed model that uses genome-wide data to model the pair-wise relatedness among the individuals.¹⁵

Sequenom Replication and Confirmation of Imputation Accuracy

SNPs with $p < 1 \times 10^{-5}$ in the additive model or $p < 5 \times 10^{-7}$ in the genotypic model were directly genotyped in the discovery set to confirm imputation accuracy and in the replication sample set to confirm the associations using two Sequenom iPLEX assays. Five SNPs looked unreliable after inspection of the cluster plots, leaving 37 SNPs in the analysis (the cluster plot for the most significant SNP is shown in Figure S8). All of these SNPs had a call rate greater than 95% and the genotype distribution among controls obeyed Hardy-Weinberg equilibrium ($p > 0.05$). After removing the samples that were originally excluded from the discovery and ImmunoChip analyses, 102 and 80 samples were removed because of the low call rate (<80%) and 7 and 9 samples because of the mismatching gender from the first and second multiplexes, respectively. This left 1,514 case subjects (418 pneumococcal cases) and 2,642 control subjects in the discovery sample set and 407 case subjects (103 pneumococcal cases) and 1,333 control subjects in the replication analyses. Genotyping of these two iPLEXes was performed at the Wellcome Trust Sanger Institute. The func-

tional SNP rs334 in *HBB* failed the initial assay design, and was therefore genotyped separately using a Sequenom iPLEX at the Wellcome Trust Centre for Human Genetics, University of Oxford. The QC measures described above were applied to these samples, leaving 1,360 case subjects and 2,644 control subjects in the discovery set and 389 case subjects and 1,312 control subjects in the replication set.

Only the samples that were included in either the discovery set or ImmunoChip replication set were included in the final analysis to allow inclusion of the first two PCs in logistic regression analysis using PLINK¹⁶ and to model the pair-wise relatedness in a linear mixed model. The combined statistics for the discovery and replication samples were obtained using fixed effects meta-analysis in GWAMA.¹⁷ The replication dataset had 80% power to detect an association ($p < 0.05$) with a common SNP (MAF 0.20) that has an effect size ≥ 1.3 , but for more rare SNPs (MAF = 0.05), an effect size ≥ 1.54 was required (see Figure S9). Therefore, we did not have sufficient statistical power to reliably replicate associations with modest effect sizes.

Approaches to Handle Relatedness

The SNPs chosen for replication were also analyzed via a linear mixed model¹⁵ that uses genome-wide data to model the pair-wise relatedness among the individuals, and which also included the first two PCs as covariates, to better account for relatedness and possible population structure within the sampled individuals. This was done by including all relatives and also by including only distantly related individuals ($r < 0.2$).

We further assessed whether the sample set with pneumococcal infection includes more pairs of close relatives than other bacteremia case subjects or than control subjects. This was assessed by comparing the observed number of relative pairs with estimated $r > 0.025$ among the pneumococcus case subjects to an equal-sized set of the rest of the case or control subjects that are matched with respect to manual clustering (Figure S11) by resampling 100,000 datasets.

Bayesian Model Comparisons

To compare models of the similarity of effect across bacterial species at identified disease-associated loci, we took a Bayesian approach (for a similar approach, see Band et al.¹⁸ and Bellenguez et al.¹⁹). The likelihood function is based on multinomial regression with strata corresponding to the control subjects and each of the seven most common bacterial subgroups (Figure 2; Table S3). Case subjects infected with more than one of these seven different bacterial species (2.1% of case subjects) are included in the analysis for each group.

The parameters of interest are the genetic effect sizes (b_k , $k = 1, \dots, 7$) on a log-odds scale for each of the case cohorts. We first find maximum likelihood estimates (with the corresponding observed information matrix) by including two PCs as covariates in the model, and then compute approximate Bayes factors using a multivariate normal approximation to the likelihood and the prior. The models are defined by prior distributions on the parameters b_k :

NULL: $b_k = 0$ for all $k = 1, \dots, 7$, i.e., no effects, all case groups are like the control group.

SAME: $b_k \sim N(0, 1)$ and $\text{cor}(b_i, b_j) = 1$ for all pairs $i \neq j$, i.e., each b_k is the same.

REL: $b_k \sim N(0, 1)$ and $\text{cor}(b_i, b_j) = 0.96$ for all pairs $i \neq j$, i.e., b_i and b_j are correlated but not necessarily the same.

Additional models are defined after inspection of the observed association at each locus for each pathogen. Bacterial species hypothesized to be associated with a given locus are assumed to have the same non-zero effect with a prior of $N(0,1)$, whereas for other pathogens the effect is 0.

Quantification of lincRNA Expression in Primary Immune Cell Subsets

Previous reports suggest that AC011288.2 encodes a lincRNA and is expressed in white blood cells and placental tissue. To identify which leukocyte population this lincRNA is expressed in, we isolated monocytes, B cells, and natural killer (NK) cells from consenting healthy adult European-ancestry donors using magnetic activated cell sorting (MACS, Miltenyi), as previously described.²⁰ In addition, we isolated granulocytes (predominantly neutrophils) using Polymorphoprep (Allere) according to the manufacturer's instructions from eight individuals. The purity of cell subsets after cell separation was assessed by flow cytometry and was >90% in a representative sample. Viability after sorting was assessed by the Trypan Blue dye exclusion method and observed to be >95% in all cases. Total RNA was extracted with the RNeasy mini kit (QIAGEN) or TRIzol (Life Technologies) according to the manufacturer's instruction (QIAGEN). Total RNA was quantified by Nanodrop and Bioanalyzer for a subset according to the manufacturers' instruction (Bioanalyzer RNA 6000 Nano kit, Agilent).

To quantify levels of lincRNA expression, we performed quantitative real-time PCR (qPCR) using a relative quantification method. Beta-Actin (ACTB) was selected as a reference gene based on previous reports of its stable expression in neutrophils. Single-strand complementary DNA was synthesized by reverse transcription with the SuperScriptIII First-Strand Synthesis System (Invitrogen). Primers specific to each of the two reported transcripts for the lincRNA AC011288.2 gene were designed: AC011288.2-001 (for, 5'-GTCAGAAGCGGGTTCAAAG-3'; rev, 5'-TTTAATTCTTGAGTTCTGCAGGC-3') and AC011288.2-002 (for, 5'-GATGCTAAGCCTGGAAACCC-3'; rev, 5'-TCCAGCTTCTATTTCCAGAGG-3'). In addition we designed primers to AC006000.5 (for, 5'-ACTCCACGTCCCACAGATAC-3'; rev, 5'-TGACAGAGTGAGACCCTGTG-3') but consistent with previous reports that observed no expression in leucocytes, we did not identify any individuals that expressed this transcript and do not describe it further. To avoid potential amplification of genomic DNA, primers were designed to span exons. qPCR was performed using SYBR Green Supermix (BioRad) on a CFX96 Real-Time PCR Detection System (Bio-Rad). Reactions were run in duplicate with 1 cycle at 95°C (10 min), followed by 42 cycles consisting of denaturation at 95°C (10 s), annealing at 58°C (20 s), and extension at 72°C (20 s). Detection of the fluorescent products was carried out at the end of the 72°C extension period. To confirm amplification specificity, the PCR products were subjected to a melting curve analysis and agarose gel electrophoresis. Detection of a fluorescent product after cycle 38 ($C_t = 38$) was considered evidence of expression beneath the confident detection limit based on careful inspection of the melting curves and agarose gel electrophoresis results. Therefore, if C_t values of greater than 38 were obtained, the C_t value was re-assigned to 38, so as to conservatively estimate the highest level of expression beneath the detectable level. On average, the C_t value for AC0011288.2-001 was 33 cycles in neutrophils. Relative gene transcript levels were determined by the $[\Delta\Delta C_t]$ method expressed relative to ACTB. Compari-

sons of log10 transformed relative expression levels were made using a non-parametric Mann-Whitney test in GraphPad Prism.

Results

Genome-wide Association Results of Directly Genotyped Discovery and Immunochip Replication Sets

We identified several suggestive associations in the directly genotyped discovery data both in pneumococcal bacteremia and bacteremia overall analyses in Kenyan children (Table S4), but none of the SNPs reached established criteria for identifying novel associations ($p < 5 \times 10^{-8}$) in a combined analysis after replication.

Genome-wide Association Results of Bacteremia Caused by *Streptococcus pneumoniae* after Imputation and Replication

After genome-wide imputation and quality control, nearly 10 million autosomal SNPs were included in the association analyses of pneumococcal bacteremia (Figure S10). In this analysis of 429 case and 2,677 control subjects, 17 SNPs in a single region on chromosome 7 were associated with disease at a level exceeding genome-wide significance ($p < 5 \times 10^{-8}$), with the peak of that association observed at rs140817150 (p imputed = 7.25×10^{-9} ; OR = 2.74) (Figures S10 and 1). This novel associated region includes two overlapping long intergenic non-coding RNA (lincRNA) genes: AC00600.5 and AC011288.2. The association at rs140817150 was confirmed by direct genotyping (p discovery = 3.58×10^{-7} ; OR = 2.39) and replication (p = 1.16×10^{-3} ; OR = 2.72), resulting in a combined OR estimate of 2.47 (95% CI 1.84–3.31) and p value of 1.69×10^{-9} (see Table S5 for a list of all suggestive associations in the analysis of pneumococcal bacteremia and Table S6 for the comprehensive list of associated SNPs in the chromosome 7 top associated region). Direct genotyping of the top imputed SNPs confirmed that imputation was generally accurate (average concordance between imputed and directly genotyped genotypes was 98.3%). In order to protect against spurious associations due to possible cryptic relatedness, the SNPs chosen for replication were also analyzed using the mixed model approach (rs140817150; p combined = 1.5×10^{-10} , OR = 2.66; Table S7) and by stratifying individuals on the basis of genetic background across the main ethnic groups (rs140817150; p discovery = 1.5×10^{-7} , OR = 2.54; Figure S11). By either approach, the evidence for association remained strong. The pneumococcal case subjects do not have more close relatives than the control subjects in any of the four ancestry groups ($p \geq 0.22$). When compared to other case subjects, they show elevated levels of relatedness ($p < 0.05$) in group 1 only, which does not contribute strongly to the observed association signal (Figure S11). After conditioning on the top SNP, no associations were detected with $p < 10^{-4}$ in the region.

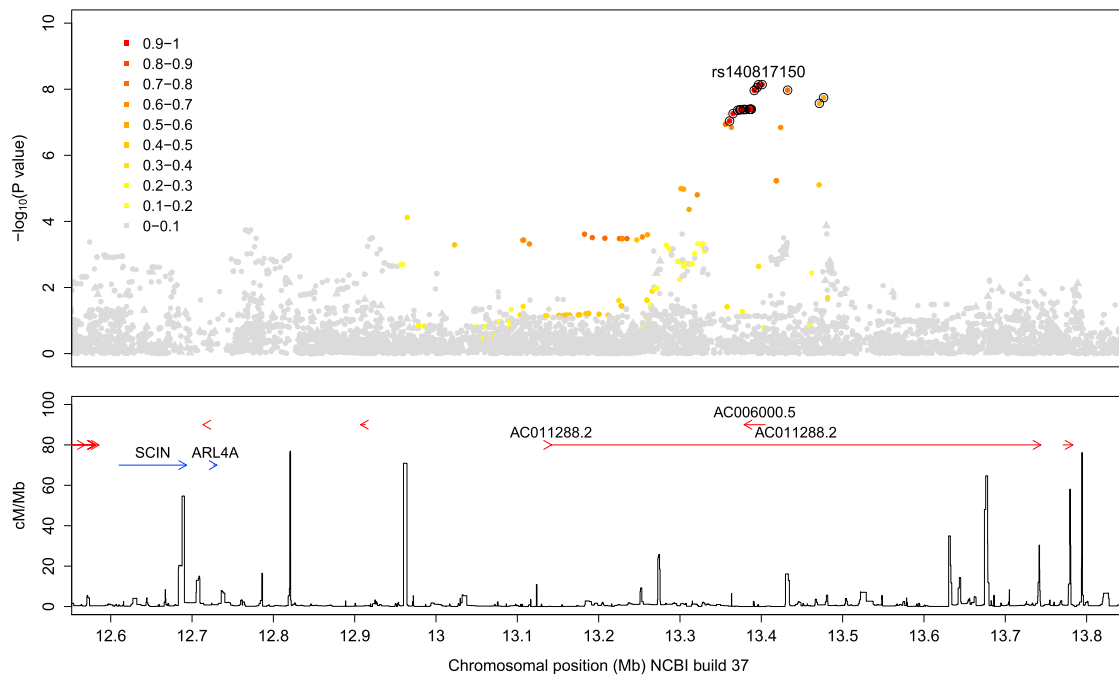


Figure 1. Signal of Association around rs140817150 in the Discovery Analysis of Pneumococcal Bacteremia

Imputed SNPs are shown as circles and directly genotyped SNPs as triangles with colors indicating the correlation (r^2 in 1000 Genomes data) with rs140817150. A set of SNPs that contains the causal SNP with greater than 95% probability is ringed with circles. Annotated genes (blue) and lincRNAs (red) are shown in the bottom panel along with the fine-scale recombination rate.

Genome-wide Association Results of Bacteremia Overall after Imputation and Replication

In addition to the analysis of a more homogeneous phenotype, pneumococcal bacteremia, all-cause bacteremia overall (Figure S12) was also analyzed. Table S8 summarizes loci with the strongest evidence of association in bacteremia overall after imputation, including the replication results. The only genome-wide significant ($p < 5 \times 10^{-8}$) association signal that replicated was under the genotypic model, which allows independent effects on risk for homozygotes and heterozygotes. It revealed a strong association at the previously identified *HBB* locus (rs113892119, $p = 5.08 \times 10^{-13}$). The region of association included the functional rs334 polymorphism ($p = 1.33 \times 10^{-10}$) that leads to the production of hemoglobin S (HbS)²¹ and is located 25.6 kb upstream from the most associating SNP rs113892119.

As previously described, rs334 was associated with susceptibility among homozygotes²² (HbSS versus HbAA; directly genotyped combined $p = 2.66 \times 10^{-12}$, OR = 4.9) and with protection from bacteremia among heterozygotes⁷ (HbAS versus HbAA; directly genotyped combined $p = 4.67 \times 10^{-3}$, OR = 0.77). These same effects are seen in the most common bacterial subgroups (Figure S13).

Bayesian Model Comparison Results of rs140817150 and rs334 Associations with Common Causes of Bacteremia in Kenyan Children

Although the association with rs140817150 was discovered in the pneumococcal bacteremia analysis, we were able to utilize the all-cause bacteremia data at this locus to assess

its effect on susceptibility to bacteremia caused by other species (Figure 2). To assess whether the data were consistent with the same effect among case subjects with different species of bacteremic pathogen, we compared models via a Bayesian approach (Figure 2). Assuming all models to be equally likely a priori, the most probable model is the one in which the susceptibility is confined to pneumococcus, *Acinetobacter* species, and *Haemophilus influenzae*. Removing the pneumococcal group, from which the association was ascertained, weakened the evidence for effect heterogeneity. The same effect in all subtypes was found to be the most probable model for rs334 association (heterozygote risk and homozygote protection) in *HBB* (Figure S13).

lincRNA Expression in Primary Immune Cell Subsets

We assessed AC011288.2 RNA expression in the major leukocyte cell subsets and observed expression only in neutrophils. Expression levels were below the detection limit in monocytes, B cells, and natural killer (NK) cells (Figure 3). To verify that expression of this transcript is constitutive in neutrophils, we measured expression in an additional 75 donors, recruited in a separate study,²³ and observed detectable expression in all 75 donors. We did not observe AC00600.5 expression in any leukocyte subsets.

Discussion

We report here a GWAS of bacteremia susceptibility, which is one of the few large-scale GWASs conducted in an

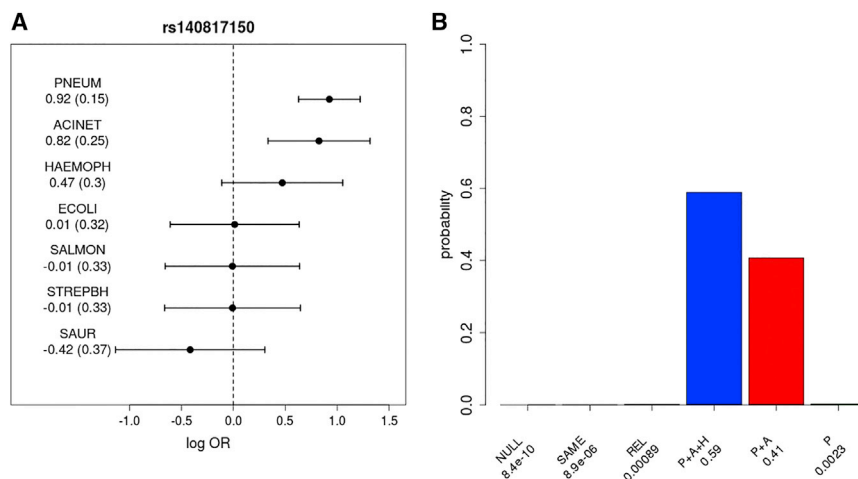


Figure 2. rs140817150 lincRNA Association with the Main Bacterial Infections

(A) Log transformed combined odds ratios and 95% confidence intervals of directly genotyped discovery and replication samples. The dotted line represents the log OR of 0 (OR of 1; no difference between case and control subjects). The values of point estimates and standard errors (in parentheses) are also given. Bacterial infection abbreviations are as follows: PNEUM, *Streptococcus pneumoniae* (pneumococcus); ACINET, *Acinetobacter* species; HAEMOPH, *Haemophilus influenzae*; ECOLI, *Escherichia coli*; SALMON, *Salmonella* (non-typhoidal); STREPBH, *Streptococcus beta hemolytic*; SAUR, *Staphylococcus aureus*.

(B) The posterior probabilities on the models of association: no effect in any subtype (NULL), same effect in all subtypes (SAME),

related effects across subtypes (REL), or the same non-zero effect only in PNEUM, ACINET, and HAEMOPH (P+A+H), in PNEUM and ACINET (P+A), or in PNEUM (P). (See [Subjects and Methods](#).) Models are a priori assumed to be equally likely. Bayes factors, which compare the evidence (marginal likelihood) between any pair of models, can be calculated as the ratio of the posterior probability assigned to each model as reported under each bar of the plot.

African population to date. We identified an association between polymorphisms in two overlapping long intergenic non-coding RNA (lincRNA) genes (AC00600.5 and AC011288.2) and pneumococcal bacteremia, the most common cause of bacteremia in our study set. Although immune responses and genetic variants affecting bacteremia susceptibility are likely to be at least partially pathogen specific, we also analyzed the bacteremia overall dataset to identify more universal risk factors. The only genome-wide significant hit for bacteremia overall was in a previously reported gene, *HBB*, including the well-known rs334 polymorphism associated with the production of sickle hemoglobin.²¹

The lincRNA risk allele at rs140817150 is derived (as reported on dbSNP) rather than ancestral, its frequency is low (2.7% in control subjects, 6.4% in pneumococcal bacteremia case subjects), and according to the 1000 Genomes project data (phase 3), it is polymorphic only in African populations. Consistent with the local recombination landscape and with the expectation that low-frequency derived alleles are relatively young, SNPs in linkage disequilibrium with rs140817150 extend over 500 kb (Figure 1). However, Bayesian analysis of the region of association²⁴ in the imputed data suggests there is greater than 95% probability that one of the most associated SNPs (circled in Figure 1) is the causal variant, assuming there is a single causal variant, and it is imputed accurately in our dataset.

The association peak is located in the introns of two separate long intergenic non-coding RNAs (lincRNAs), annotated as AC011288.2 and AC006000.5. The importance of lincRNAs as key regulators of gene expression has only recently been recognized.^{25–27} It has been estimated that the human genome includes at least 10,000 lincRNAs but only a fraction of these has a known function.^{25–27} A recent study aiming to catalog the function of more

than 8,000 human lincRNAs reported that lincRNA expression is significantly more tissue specific than expression of protein-coding genes.²⁸ AC006000.5 is listed in the catalog but it is not expressed in any of the studied tissues, whereas AC011288.2 is reported to be expressed only in placenta and white blood cells out of 24 different tissues and cell lines studied. We assessed AC011288.2 RNA expression in leukocyte cell subsets and observed expression only in neutrophils, a cell type that is known to play a major role in pneumococcal clearance.^{29,30} These results provide an important direction for future functional investigations. Neutrophils express many antimicrobial peptides and proteins that confer both universal and pathogen-specific host response,^{31,32} and it has been shown that absolute neutrophil count is an independent predictor of pneumococcal bacteremia in febrile children.³³

The closest protein-coding genes surrounding the association signal are *ARL4A* and *ETV1* but there is no evidence that the associating lincRNAs regulate these two genes. However, data from a previous expression quantitative trait locus (eQTL) study²⁰ suggest that there are some SNPs in the associating region that function as eQTLs in monocytes (rs1432496) and B cells (rs2568633) for *PHF14* (PHD finger protein 14), a transcription factor that downregulates *PDGFRα* expression.³⁴ However, neither is correlated with our most-associated SNP ($r^2 < 0.01$ in 1000 Genomes data). Although the role of lincRNAs in human infections is unknown, recent mouse studies have indicated that some lincRNAs can act in immune cells to regulate host susceptibility to bacterial and viral infections.^{35,36}

Using the GWAS approach, we have identified an association between a genetic variation in a lincRNA gene and pneumococcal bacteremia. Furthermore, we have confirmed a previously reported association between *HBB* and bacteremia overall,²² with homozygotes associated with strong susceptibility but heterozygotes associated

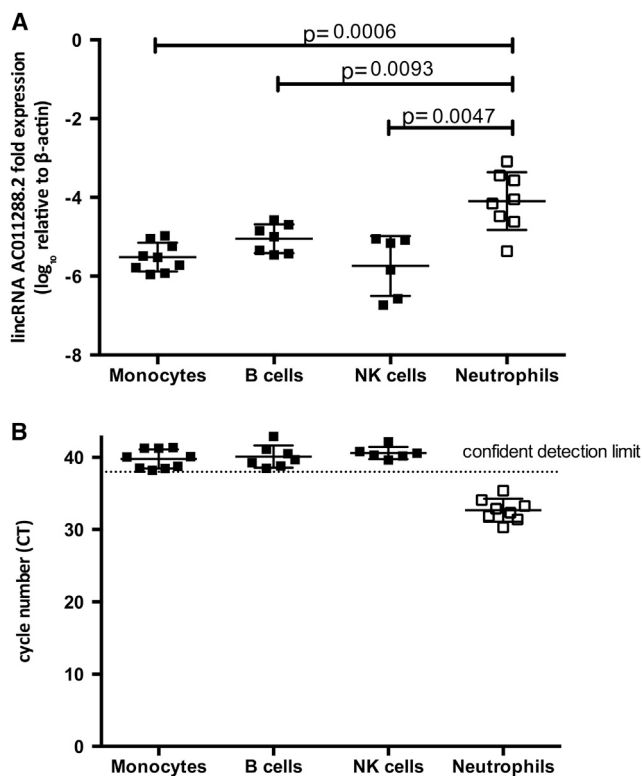


Figure 3. lincRNA AC011288.2 Expression Measured in Neutrophils, Monocytes, B Cells, and NK Cells

(A) Quantitative PCR of AC011288.2-002 in primary leucocyte subsets. To conservatively estimate the highest level of expression beneath the detectable level, CT values greater than 38 were re-assigned to 38, and normalized to β-actin expression. p values denote the significance of the relative expression levels of AC011288.2-001 in neutrophils compared to other cell types (Mann-Whitney test).

(B) Unadjusted cycle number of amplification is shown. Filled squares denote detection of a fluorescent product after cycle 38 (Ct = 38), the limit of confident detection being based on careful inspection of the melting curves. Similar results for AC011288.2-001 were obtained (data not shown).

with protection. At both associated loci, the disease-associated alleles are rare in individuals without African ancestry (monomorphic in 1000 Genomes Project data in other than African populations) and exert a large effect on the likelihood of developing bacteremia. These associations have not been reported by earlier GWASs of related phenotypes, which is unsurprising because the populations under study have been of European descent and differences in phenotypes are still substantial.^{37,38} The reported SNPs in *FER* (MIM: 176942) that was recently associated with outcome from sepsis due to pneumonia³⁷ or in *CFH* (MIM: 134370) and *CFHR3* (MIM: 605336) that have been associated with meningococcal disease³⁸ did not show any evidence of association in the current study ($p > 0.05$). Given the likely importance of host-pathogen molecular interactions in bacteremia susceptibility, it is plausible that the effect of a risk allele will be dependent on bacterial species. Our data on the lincRNA locus provide initial evidence for this at the bacterial species level, and motivate approaches that

stratify host genetic associations by pathogen species, serotype, or genotype. Understanding the molecular mechanisms leading to the doubled risk of pneumococcal bacteremia associated with this allele could provide new clues in the pressing search for new therapeutic targets.

Accession Numbers

Genotype data will be made available by the WTCCC access process, via the European Genotype Archive under the accession number EGAS00001001756.

Supplemental Data

Supplemental Data include 13 figures and 8 tables and can be found with this article online at <http://dx.doi.org/10.1016/j.ajhg.2016.03.025>.

Consortia

The Kenyan Bacteraemia study group consists of the following individuals. Principal investigators are Adrian V.S. Hill (Chair), Thomas N. Williams, J. Anthony G. Scott, and Stephen J. Chapman. Key personnel are Anna Rautanen, Tara C. Mills, Kirk A. Rockett, Anne W. Ndungu, Vivek Naranbhai, Alex W. Macharia, Sophie Uyoga, Carolyne Ndila, Neema Mturi, Patricia Njuguna, Shebe Mohammed, James A. Berkley, Isaiah Mwangi, Salim Mwarumba, Barnes S. Kitsao, Brett S. Lowe, Susan C. Morpeth, and Iqbal Khandwalla. The Kilifi DNA Extraction Group members are Alex W. Macharia, Sophie Uyoga, Herbert Opi, Carolyne Ndila, Emily Nyatichi, Prophet Ingosi, Barnes Kitsao, Clement Lewa, Johnstone Makale, Adan Mohamed, Kenneth Magua, Mary Njoroge, Gideon Nyutu, Ruth Mwarabu, Metrine Tendwa, and Thomas N. Williams. The Kilifi Bacteraemia Surveillance Group consists of the following individuals: Ismail Ahmed, Samuel Akech, Alexander Balo Makazi, Mohammed Bakari Hajj, Andrew Brent, Charles Chesaro, Hiza Dayo, Richard Idro, Patrick Kosgei, Kathryn Maitland, Kevin Marsh, Laura Mwalekwa, Shalton Mwaringa, Charles Newton, Mwanajuma Ngama, Allan Pamba, Norbert Peshu, Anna Seale, Alison Talbert, and Thomas N. Williams.

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Web Resources

1000 Genomes, <http://www.1000genomes.org>

European Genome-phenome Archive (EGA), <https://www.ebi.ac.uk/ega>

OMIM, <http://www.omim.org/>

References

- Lillie, P.J., Allen, J., Hall, C., Walsh, C., Adams, K., Thaker, H., Moss, P., and Barlow, G.D. (2013). Long-term mortality following bloodstream infection. *Clin. Microbiol. Infect.* **19**, 955–960.
- Wyllie, D.H., Crook, D.W., and Peto, T.E. (2006). Mortality after *Staphylococcus aureus* bacteraemia in two hospitals in Oxfordshire, 1997–2003: cohort study. *BMJ* **333**, 281.
- Laupland, K.B., Svenson, L.W., Gregson, D.B., and Church, D.L. (2011). Long-term mortality associated with community-onset bloodstream infection. *Infection* **39**, 405–410.
- O'Brien, K.L., Wolfson, L.J., Watt, J.P., Henkle, E., Deloria-Knoll, M., McCall, N., Lee, E., Mulholland, K., Levine, O.S., and Cherian, T.; Hib and Pneumococcal Global Burden of Disease Study Team (2009). Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. *Lancet* **374**, 893–902.
- Sørensen, T.I., Nielsen, G.G., Andersen, P.K., and Teasdale, T.W. (1988). Genetic and environmental influences on premature death in adult adoptees. *N. Engl. J. Med.* **318**, 727–732.
- Berkley, J.A., Lowe, B.S., Mwangi, I., Williams, T., Bauni, E., Mwarumba, S., Ngetsa, C., Slack, M.P., Njenga, S., Hart, C.A., et al. (2005). Bacteremia among children admitted to a rural hospital in Kenya. *N. Engl. J. Med.* **352**, 39–47.
- Scott, J.A., Berkley, J.A., Mwangi, I., Ochola, L., Uyoga, S., Macharia, A., Ndila, C., Lowe, B.S., Mwarumba, S., Bauni, E., et al. (2011). Relation between falciparum malaria and bacteraemia in Kenyan children: a population-based, case-control study and a longitudinal study. *Lancet* **378**, 1316–1323.
- Barrett, J.C., Lee, J.C., Lees, C.W., Prescott, N.J., Anderson, C.A., Phillips, A., Wesley, E., Parnell, K., Zhang, H., Drummond, H., et al.; UK IBD Genetics Consortium; Wellcome Trust Case Control Consortium 2 (2009). Genome-wide association study of ulcerative colitis identifies three new susceptibility loci, including the HNF4A region. *Nat. Genet.* **41**, 1330–1334.
- Consortium, W.T.C.C.; Wellcome Trust Case Control Consortium (2007). Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* **447**, 661–678.
- Morris, J.A., Randall, J.C., Maller, J.B., and Barrett, J.C. (2010). Evoker: a visualization tool for genotype intensity data. *Bioinformatics* **26**, 1786–1787.
- Cortes, A., and Brown, M.A. (2011). Promise and pitfalls of the Immunochip. *Arthritis Res. Ther.* **13**, 101.
- Delaneau, O., Marchini, J., and Zagury, J.F. (2012). A linear complexity phasing method for thousands of genomes. *Nat. Methods* **9**, 179–181.
- Howie, B.N., Donnelly, P., and Marchini, J. (2009). A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet.* **5**, e1000529.
- Marchini, J., and Howie, B. (2010). Genotype imputation for genome-wide association studies. *Nat. Rev. Genet.* **11**, 499–511.
- Pirinen, M., Donnelly, P., and Spencer, C.C. (2013). Efficient computation with a linear mixed model on large-scale data sets with applications to genetic studies. *Ann. Appl. Stat.* **7**, 369–390.
- Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J., and Sham, P.C. (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575.
- Mägi, R., and Morris, A.P. (2010). GWAMA: software for genome-wide association meta-analysis. *BMC Bioinformatics* **11**, 288.
- Band, G., Le, Q.S., Jostins, L., Pirinen, M., Kivinen, K., Jallow, M., Sisay-Joof, F., Bojang, K., Pinder, M., Sirugo, G., et al.; Malaria Genomic Epidemiology Network (2013). Imputation-based meta-analysis of severe malaria in three African populations. *PLoS Genet.* **9**, e1003509.
- Bellenguez, C., Bevan, S., Gschwendtner, A., Spencer, C.C., Burgess, A.I., Pirinen, M., Jackson, C.A., Traylor, M., Strange, A., Su, Z., et al.; International Stroke Genetics Consortium (ISGC); Wellcome Trust Case Control Consortium 2 (WTCCC2) (2012). Genome-wide association study identifies a variant in HDAC9 associated with large vessel ischemic stroke. *Nat. Genet.* **44**, 328–333.
- Fairfax, B.P., Makino, S., Radhakrishnan, J., Plant, K., Leslie, S., Dilthey, A., Ellis, P., Langford, C., Vannberg, F.O., and Knight, J.C. (2012). Genetics of gene expression in primary immune cells identifies cell type-specific master regulators and roles of HLA alleles. *Nat. Genet.* **44**, 502–510.
- Ingram, V.M. (1957). Gene mutations in human haemoglobin: the chemical difference between normal and sickle cell haemoglobin. *Nature* **180**, 326–328.

22. Williams, T.N., Uyoga, S., Macharia, A., Ndila, C., McAuley, C.F., Opi, D.H., Mwarumba, S., Makani, J., Komba, A., Ndiritu, M.N., et al. (2009). Bacteraemia in Kenyan children with sickle-cell anaemia: a retrospective cohort and case-control study. *Lancet* 374, 1364–1370.
23. Naranbhai, V., Fairfax, B.P., Makino, S., Humburg, P., Wong, D., Ng, E., Hill, A.V., and Knight, J.C. (2015). Genomic modulators of gene expression in human neutrophils. *Nat. Commun.* 6, 7545.
24. Maller, J.B., McVean, G., Byrnes, J., Vukcevic, D., Palin, K., Su, Z., Howson, J.M., Auton, A., Myers, S., Morris, A., et al.; Wellcome Trust Case Control Consortium (2012). Bayesian refinement of association signals for 14 loci in 3 common diseases. *Nat. Genet.* 44, 1294–1301.
25. Derrien, T., Johnson, R., Bussotti, G., Tanzer, A., Djebali, S., Tilgner, H., Guernec, G., Martin, D., Merkel, A., Knowles, D.G., et al. (2012). The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. *Genome Res.* 22, 1775–1789.
26. Djebali, S., Davis, C.A., Merkel, A., Dobin, A., Lassmann, T., Mortazavi, A., Tanzer, A., Lagarde, J., Lin, W., Schlesinger, F., et al. (2012). Landscape of transcription in human cells. *Nature* 489, 101–108.
27. Lee, J.T. (2012). Epigenetic regulation by long noncoding RNAs. *Science* 338, 1435–1439.
28. Cabili, M.N., Trapnell, C., Goff, L., Koziol, M., Tazon-Vega, B., Regev, A., and Rinn, J.L. (2011). Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. *Genes Dev.* 25, 1915–1927.
29. Gingles, N.A., Alexander, J.E., Kadioglu, A., Andrew, P.W., Kerr, A., Mitchell, T.J., Hopes, E., Denny, P., Brown, S., Jones, H.B., et al. (2001). Role of genetic resistance in invasive pneumococcal infection: identification and study of susceptibility and resistance in inbred mouse strains. *Infect. Immun.* 69, 426–434.
30. Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D.S., Weinrauch, Y., and Zychlinsky, A. (2004). Neutrophil extracellular traps kill bacteria. *Science* 303, 1532–1535.
31. Cederlund, A., Agerberth, B., and Bergman, P. (2010). Specificity in killing pathogens is mediated by distinct repertoires of human neutrophil peptides. *J. Innate Immun.* 2, 508–521.
32. Nguyen, Q.T., Nguyen, T.H., Ju, S.A., Lee, Y.S., Han, S.H., Lee, S.C., Kwon, B.S., Yu, R., Kim, G.Y., Lee, B.J., and Kim, B.S. (2013). CD137 expressed on neutrophils plays dual roles in antibacterial responses against Gram-positive and Gram-negative bacterial infections. *Infect. Immun.* 81, 2168–2177.
33. Kuppermann, N., Fleisher, G.R., and Jaffe, D.M. (1998). Predictors of occult pneumococcal bacteremia in young febrile children. *Ann. Emerg. Med.* 31, 679–687.
34. Kitagawa, M., Takebe, A., Ono, Y., Imai, T., Nakao, K., Nishikawa, S., and Era, T. (2012). Phf14, a novel regulator of mesenchyme growth via platelet-derived growth factor (PDGF) receptor- α . *J. Biol. Chem.* 287, 27983–27996.
35. Gomez, J.A., Wapinski, O.L., Yang, Y.W., Bureau, J.F., Gopinath, S., Monack, D.M., Chang, H.Y., Brahic, M., and Kirkegaard, K. (2013). The NeST long ncRNA controls microbial susceptibility and epigenetic activation of the interferon- γ locus. *Cell* 152, 743–754.
36. Carpenter, S., Aiello, D., Atianand, M.K., Ricci, E.P., Gandhi, P., Hall, L.L., Byron, M., Monks, B., Henry-Bezy, M., Lawrence, J.B., et al. (2013). A long noncoding RNA mediates both activation and repression of immune response genes. *Science* 341, 789–792.
37. Rautanen, A., Mills, T.C., Gordon, A.C., Hutton, P., Steffens, M., Nuamah, R., Chiche, J.D., Parks, T., Chapman, S.J., Davenport, E.E., et al.; ESICM/ECCRN GenOSept Investigators (2015). Genome-wide association study of survival from sepsis due to pneumonia: an observational cohort study. *Lancet Respir. Med.* 3, 53–60.
38. Davila, S., Wright, V.J., Khor, C.C., Sim, K.S., Binder, A., Breunis, W.B., Inwald, D., Nadel, S., Betts, H., Carrol, E.D., et al.; International Meningococcal Genetics Consortium (2010). Genome-wide association study identifies variants in the CFH region associated with host susceptibility to meningococcal disease. *Nat. Genet.* 42, 772–776.

Supplemental Data

Polymorphism in a lincRNA Associates with a Doubled Risk of Pneumococcal Bacteremia in Kenyan Children

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Supplemental Figures

Figure S1: Bacteremia GWAS workflow

Genomic DNA whole-genome amplified with GenomiPhi kit

Discovery GWAS: ~2000 cases and ~3000 controls submitted to Affymetrix for genotyping after passing the sample QC performed at Sanger

- Number of excluded samples based on QC: 581
 - o Failed at Affymetrix: 92
 - o Call rate/heterozygosity: 182 (Figure S2)
 - o Discrepancy between Sequenom and Affymetrix genotyping: 17 (5 that otherwise passed the QC)
 - o Channel: 14
 - o Gender discrepancy or unknown reported gender: 110 (32 that otherwise passed the QC)
 - o Population outlier (PCA): 186 (Figure S3)
 - o Duplicate individuals: 103 (of which 16 are intentional for QC purposes)
 - o Relatedness (Siblings removed but more distant relatives kept in the analysis, $\pi_{\text{hat}} > 0.4$): 117
- Exclusion based on phenotype: 130 (See Table S1 for further details)
- Analysis of 1536 cases and 2677 controls using the first two principal components as covariates (PLINK)
- 2000 SNPs selected for Immunochip replication:
 - o 1000: SNPs chosen based on bacteremia overall analysis
 - o 1000: SNPs chosen based on different species of bacteremic pathogen

Immunochip replication: ~500 cases and ~1500 controls

- Number of excluded samples based on QC: 212
 - o Call rate/heterozygosity: 84
 - o Discrepancy between Sequenom and ImmunoChip genotyping: 8
 - o Channel: 4
 - o Gender discrepancy: 5 (0 that would have otherwise passed the QC)
 - o Population outlier (PCA): 51
 - o Duplicate individual: 6
 - o Duplicate individual between GWAS and Immunochip: 29
 - o Relatedness (Siblings removed but more distant relatives kept in the analysis, $\pi_{\text{hat}} > 0.4$): 29
- Exclusion based on phenotype: 37 (See Table S1 for further details)
- Analysis of 434 cases and 1336 controls using the first two principal components as covariates (PLINK)
- No Genome-wide significant hits with bacteremia overall or in bacterial subgroups in the combined analysis

Imputation of the discovery set using individuals in the 1000 Genomes Project as a reference panel:

- Phasing with SHAPEIT (QCd set of samples and SNPs)
- Imputation with IMPUTE2
- Association analysis with SNPTEST2 using the score method and additive and genotypic models of association
 - o SNPs with $\text{Info} < 0.8$ and $\text{MAF} < 0.01$ excluded
- The most promising associations selected for direct genotyping with Sequenom

Direct Sequenom genotyping of the discovery and replication samples: 37 SNPs in 3 iPLEX assays

- Samples excluded based on a call rate of <80% and mismatching gender
- Discovery and replication samples analyzed separately
 - Discovery: 1514 cases and 2642 controls that passed the GWAS QC earlier
 - Discovery of rs334 (*HBB*): 1360 cases and 2644 controls
 - Replication: 407 cases and 1333 controls that earlier passed the ImmunoChip QC
 - Replication of rs334 (*HBB*): 389 cases and 1312 controls that passed the ImmunoChip QC
 - Two loci reached genome-wide significance: *HBB* in bacteremia overall (genotypic model) and a lincRNA in the pneumococcal subgroup (allelic model)
- Mixed Model analysis to better account for relatedness and underlying population structure
 - Siblings and more distantly related individuals included in the analysis:
 - Discovery: 1519 cases and 2688 controls
 - Replication: 408 cases and 1360 controls
 - All related individuals ($r > 0.2$) excluded from the analysis:
 - Discovery: 1476 cases and 2543 controls
 - Replication: 404 cases and 1311 controls

Figure S2. Sample exclusions based on call rate (x-axis) and heterozygosity (y-axis). Excluded samples are shown in red.

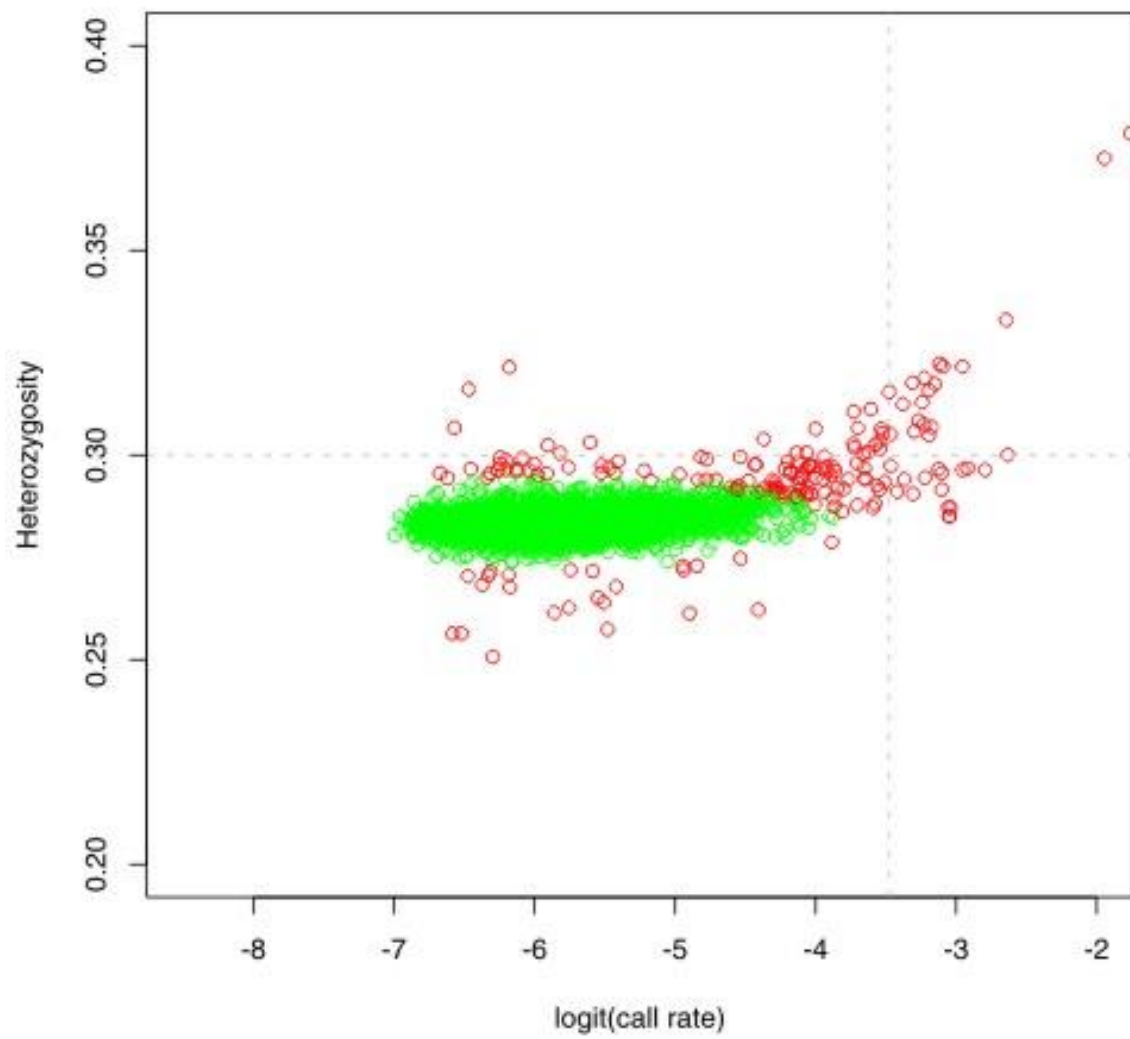
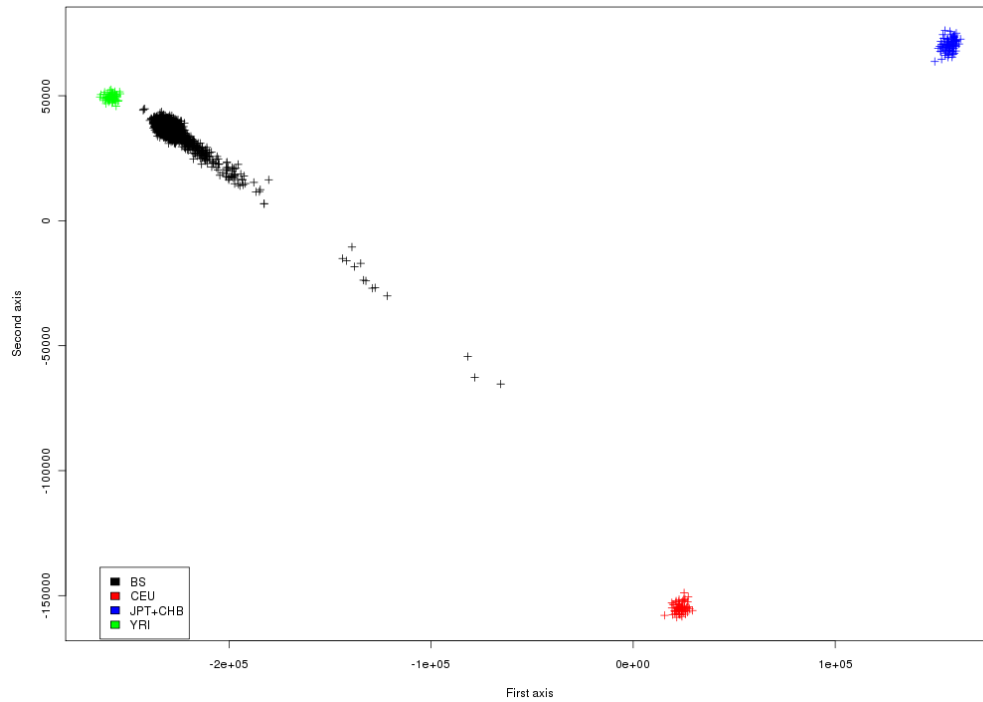


Figure S3. Principal components analysis for bacteremia cases and controls (A) analyzed together with HapMap individuals and (B) zoomed into the bacteremia cluster to show the population outlier exclusions (red). BS = bacteremia susceptibility; CEU = Utah residents with Northern and Western European ancestry from the CEPH collection; JPT = Japanese in Tokyo, Japan; CHB = Han Chinese in Beijing, China; YRI = Yoruba in Ibadan, Nigeria.

A)



B)

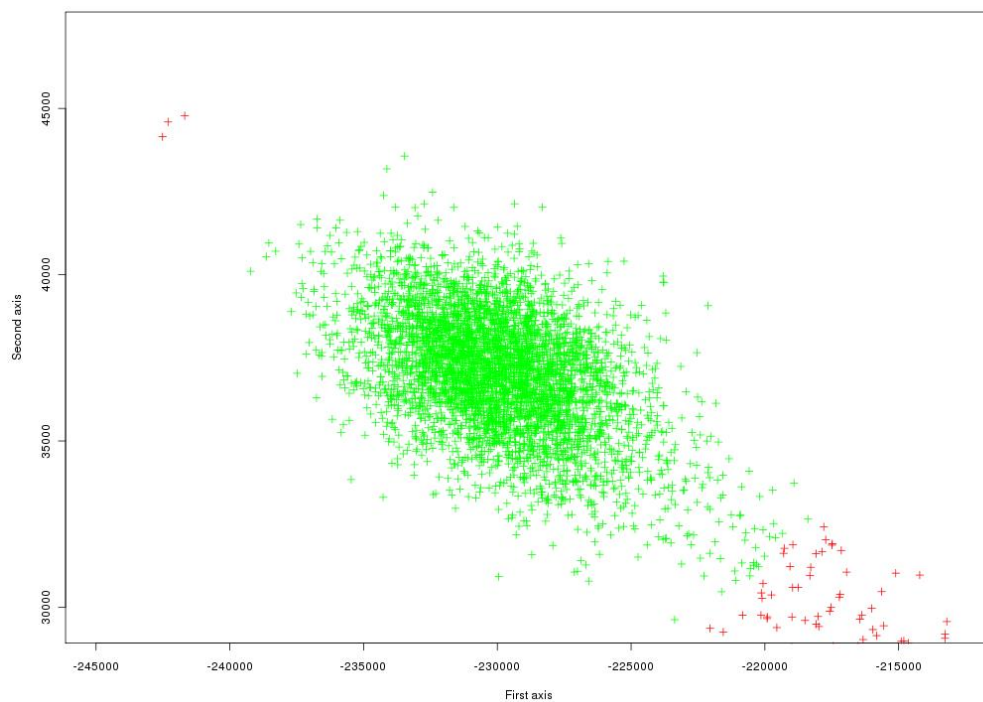


Figure S4. Relatedness shown by estimated identity by descent (IBD) across the genome for the 4000 most related sample pairs. Each vertical line represents a pairwise comparison of two individuals: red indicates two alleles IBD, blue indicates one allele IBD, and grey indicates zero alleles IBD. Thereby duplicate samples (or monozygotic twins) are shown with a full red line. Sibling pairs result in lines that are approximately one quarter red, half blue, and one quarter grey. Partly grey and partly blue lines represent sample pairs that are less related, i.e. cousins, second cousins etc. The dotted horizontal lines indicate 25% centiles.

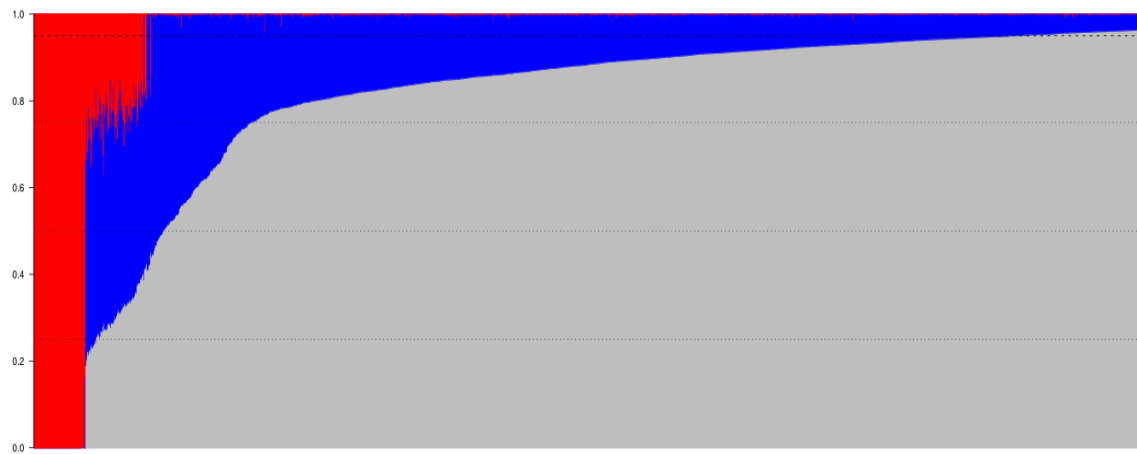
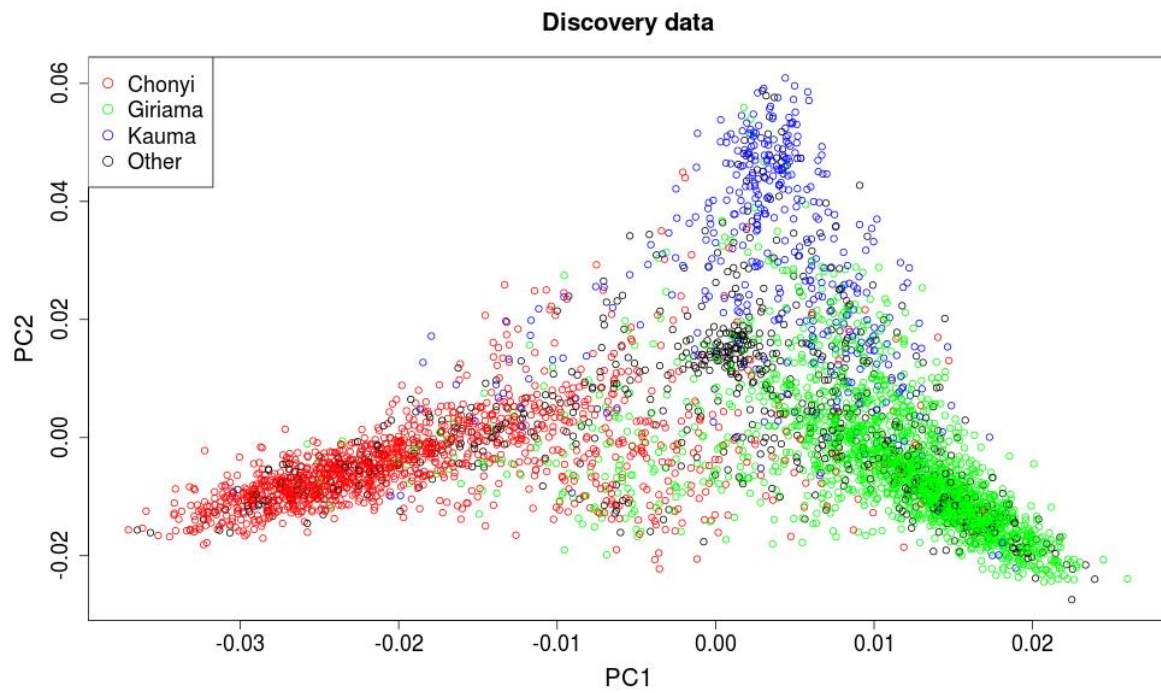


Figure S5. The first and second axes of the principal components analysis in the discovery dataset using 168,217 SNPs. Color coding represents A) reported ethnicity and B) case control status.

A)



B)

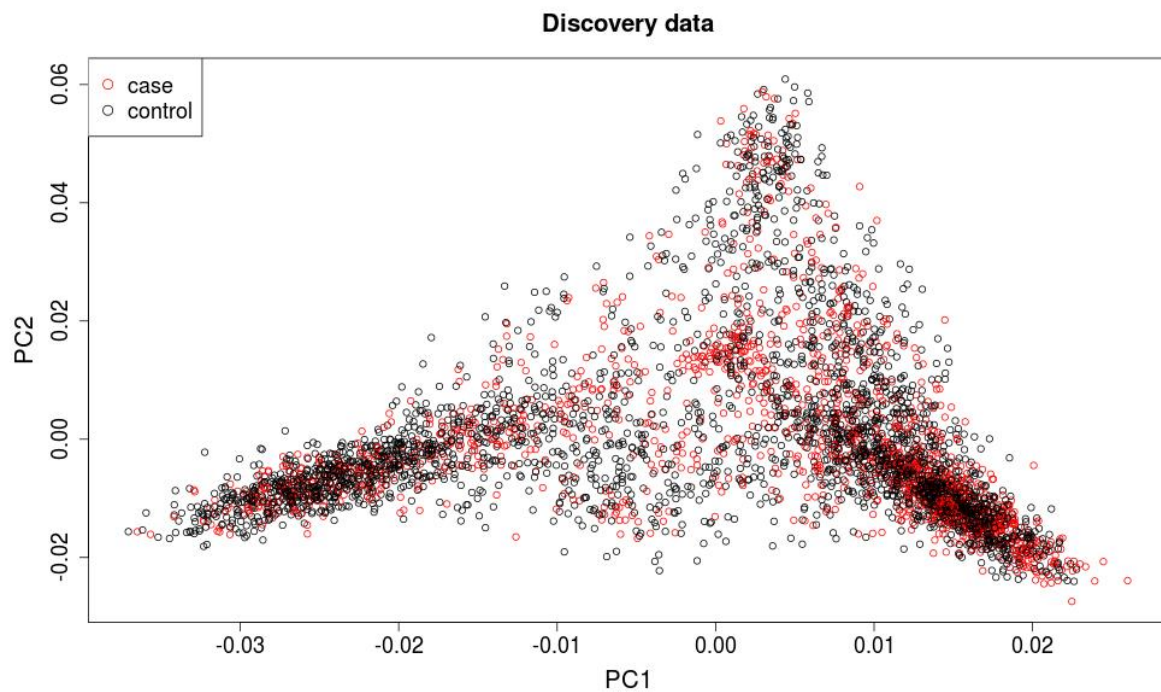
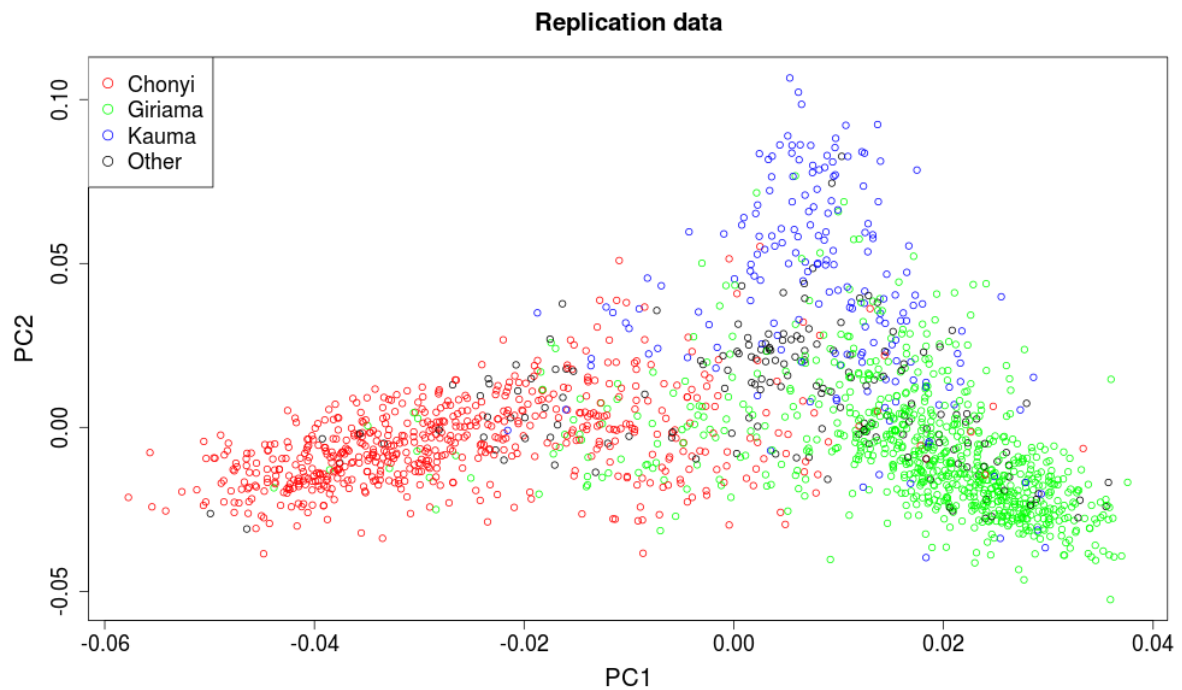


Figure S6. The first and second axes of the principal components analysis in the ImmunoChip replication dataset using 27,026 SNPs. Color coding represents A) reported ethnicity, B) case control status.

A)



B)

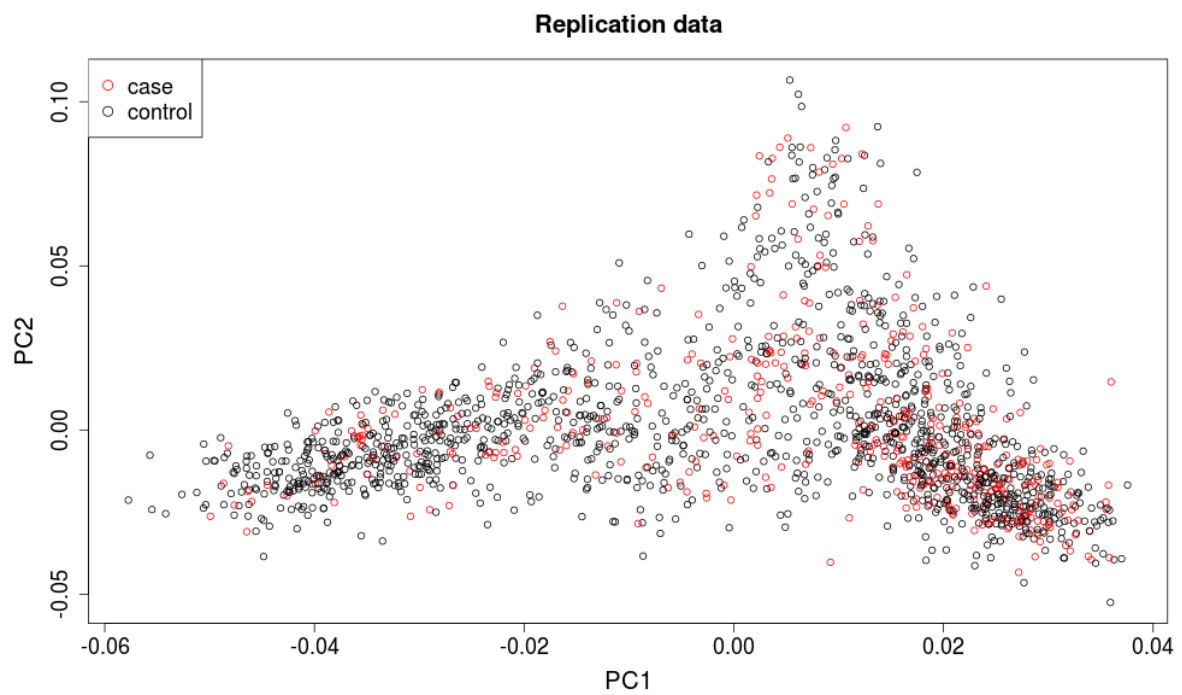


Figure S7. QQ-plots of the pneumococcal bacteremia ($\lambda=1.013$) and bacteremia overall ($\lambda=1.043$) analyses after imputation and data cleaning. Association analyses were performed with SNPTTEST2 (additive model). After the SNPTTEST analysis, every SNP with a minor allele frequency < 0.02 , info < 0.8 , or Hardy-Weinberg equilibrium $P < 1 \times 10^{-10}$ were excluded.

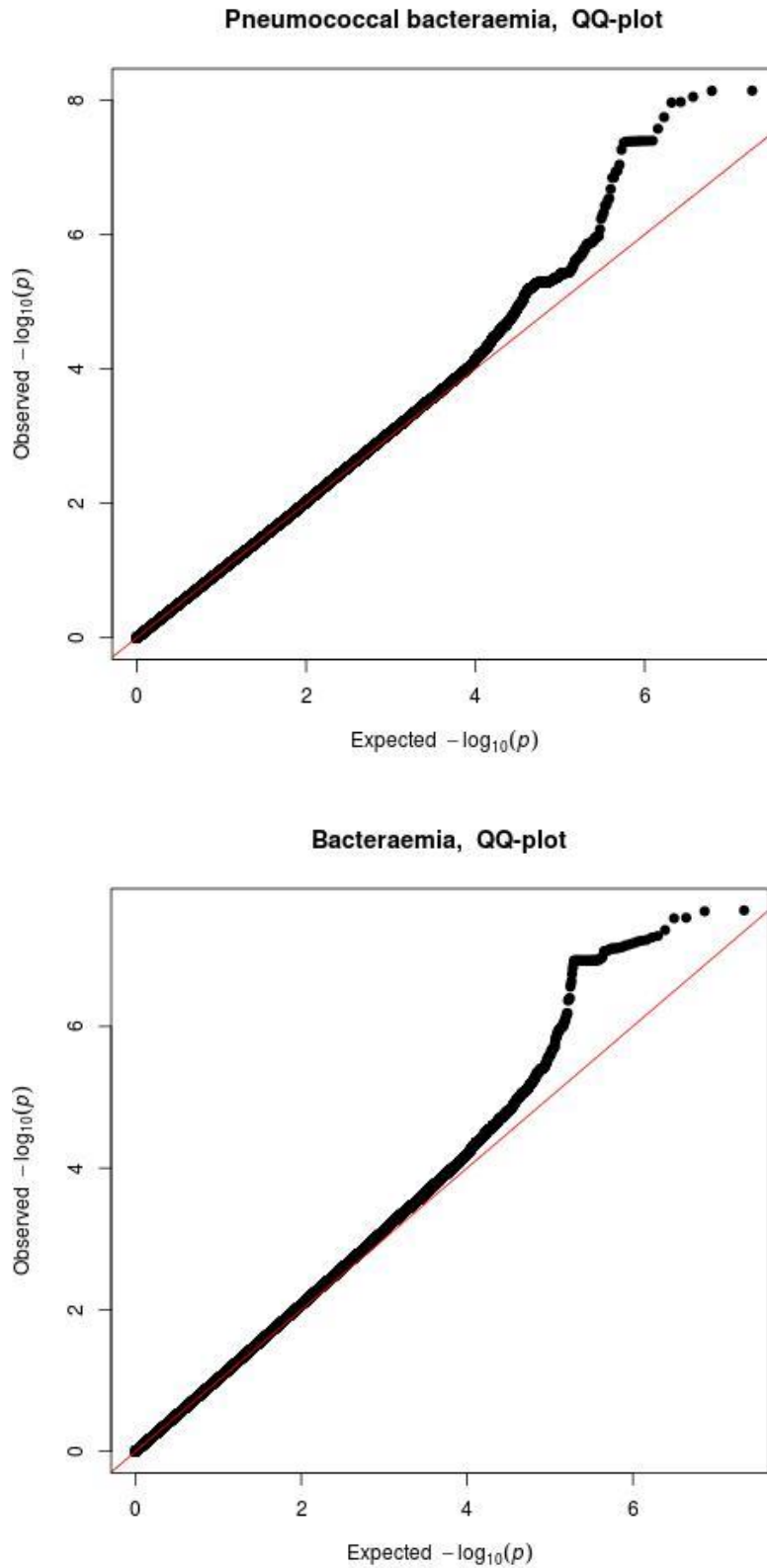


Figure S8. Sequenom cluster plot for the rs140817150 SNP (chr7 lincRNA, pneumococcal bacteremia).

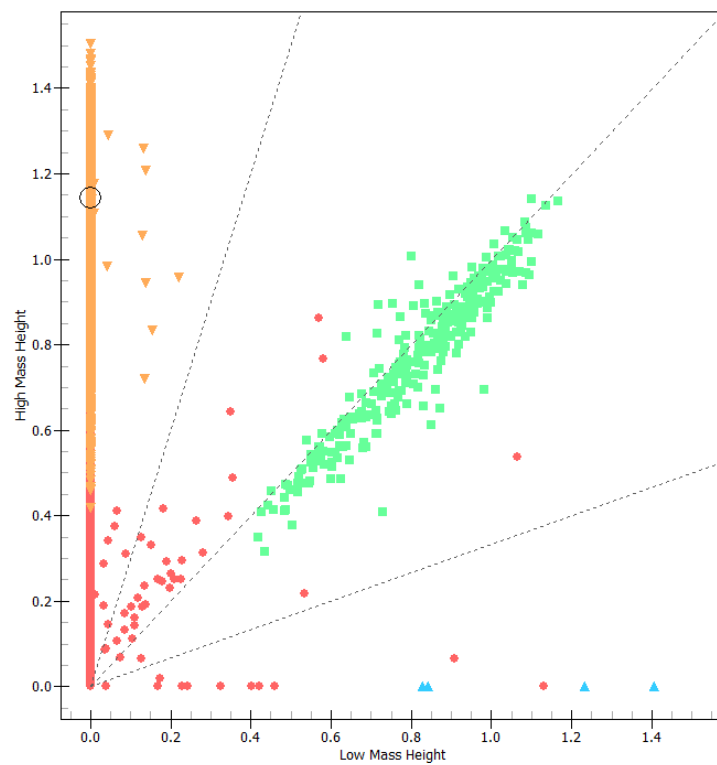


Figure S9. Statistical power to detect an association with a p-value of 0.05 in the replication data set (434 cases and 1336 controls) with varying odds ratio and minor allele frequency (MAF) as indicated.

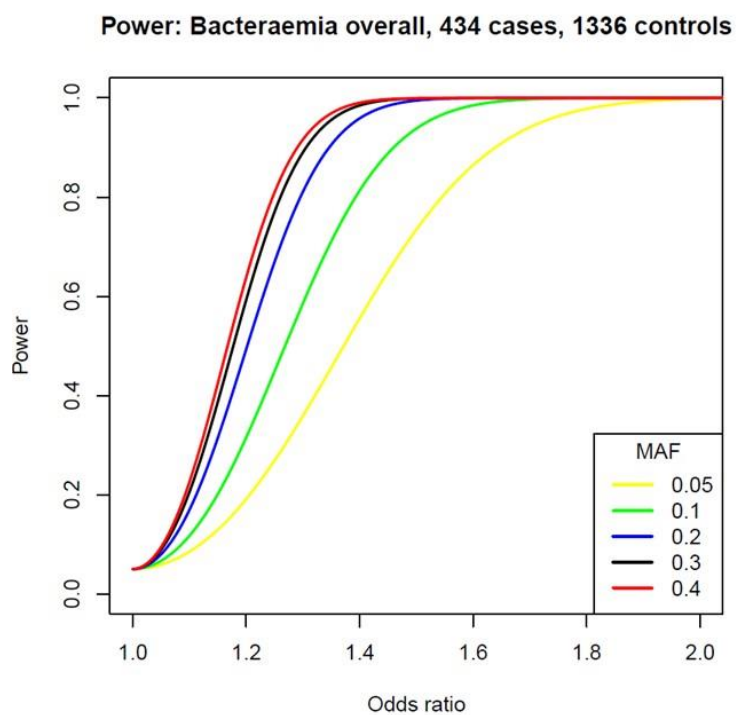


Figure S10. Manhattan plot for the pneumococcal bacteremia discovery analysis (additive model) after imputation. SNPs with minor allele frequency > 2% and info value > 0.9 are included (9,337,574 SNPs).

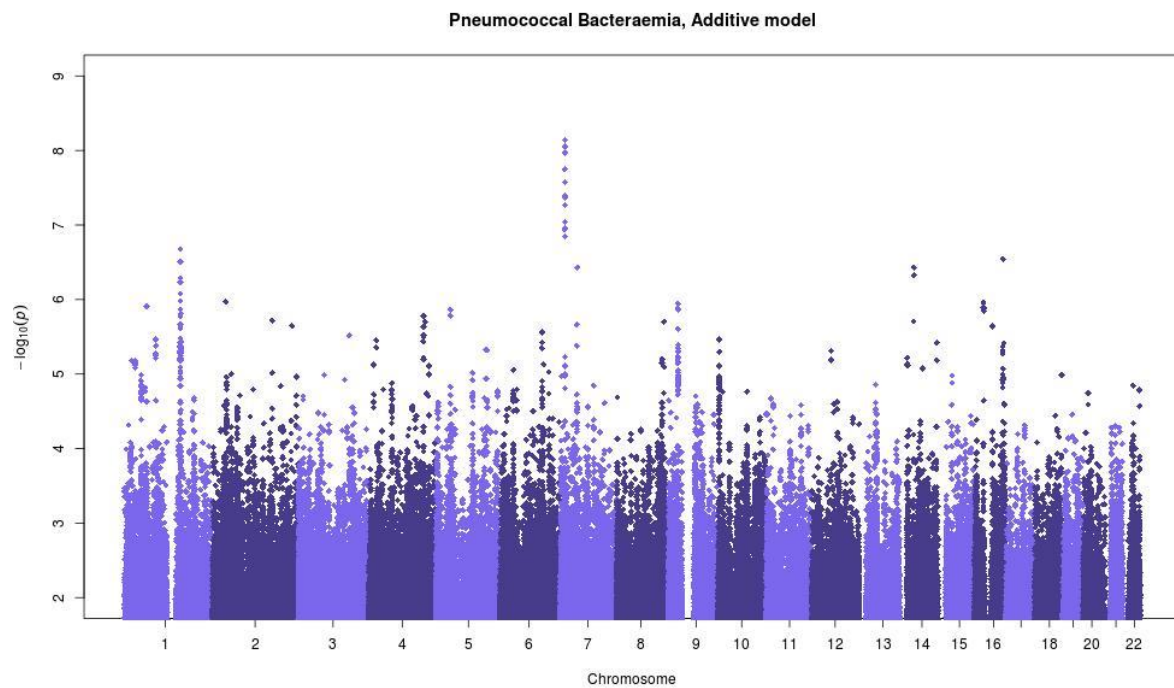
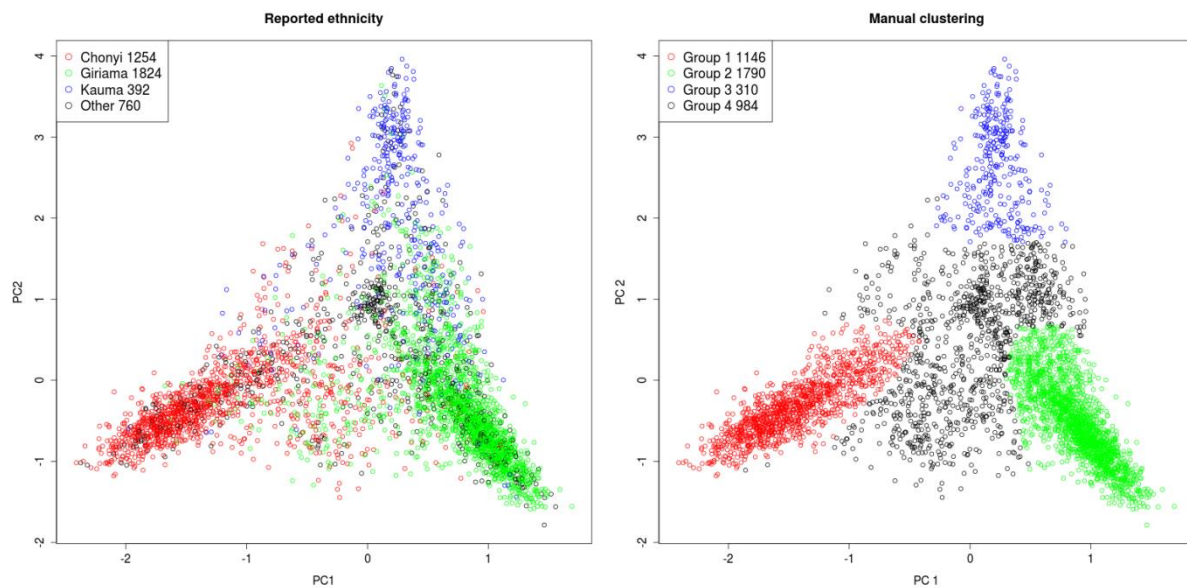


Figure S11. rs140817150 lincRNA and rs334 *HBB* associations with pneumococcal bacteremia and bacteremia overall, respectively, in the discovery data set stratified by the main ethnic groups.

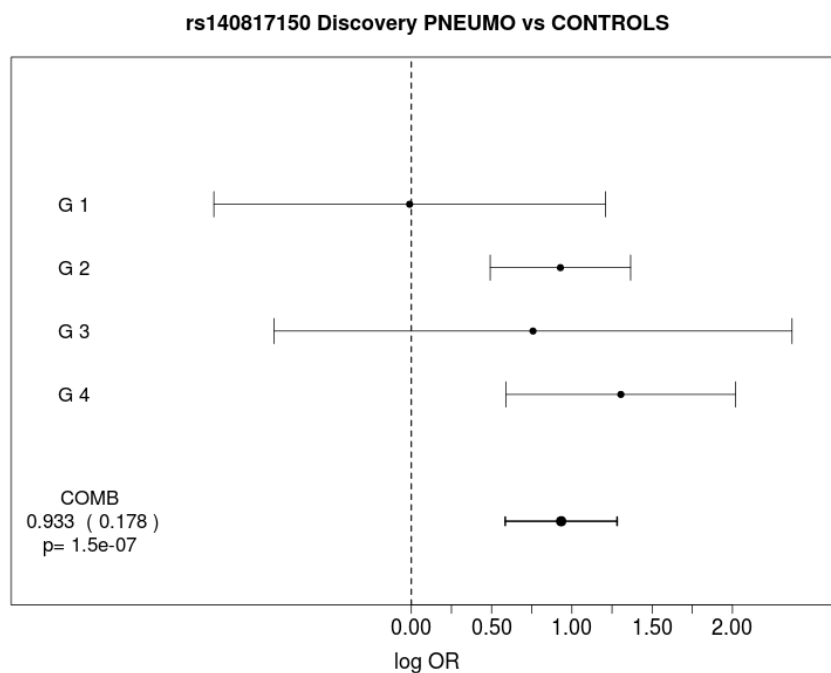
A) Left panel: The three largest ethnic groups with the first two principal components of genetic structure. Right panel: Manual clustering of the individuals into four groups reflecting reported ethnicities. Sizes of groups are in the legend.

B) Log odds-ratios separately for the four groups defined in the right panel of (A), and their combined estimate.

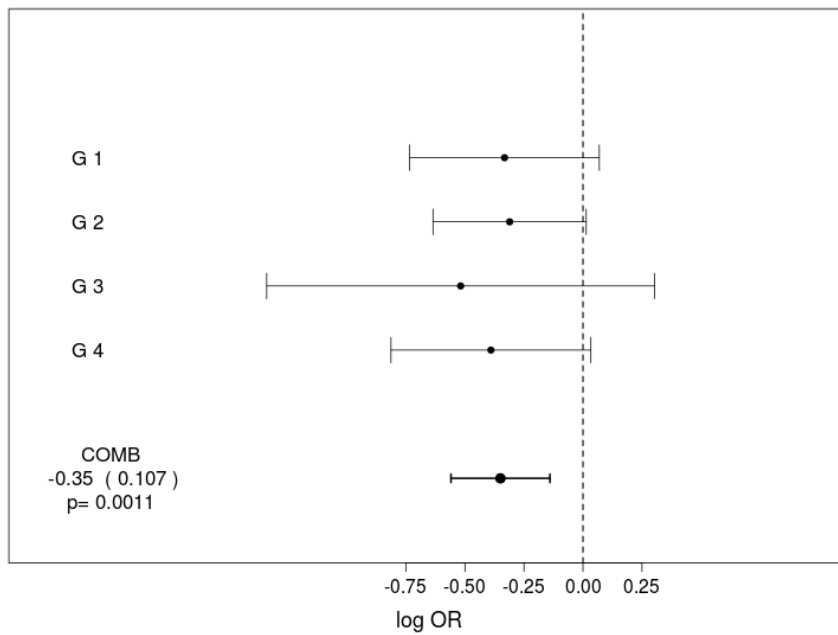
A)



B)



rs334 Discovery HbAS vs HbAA, ALL BACTERAEMIA vs CONTROLS



rs334 Discovery HbSS vs HbAA, ALL BACTERAEMIA vs CONTROLS

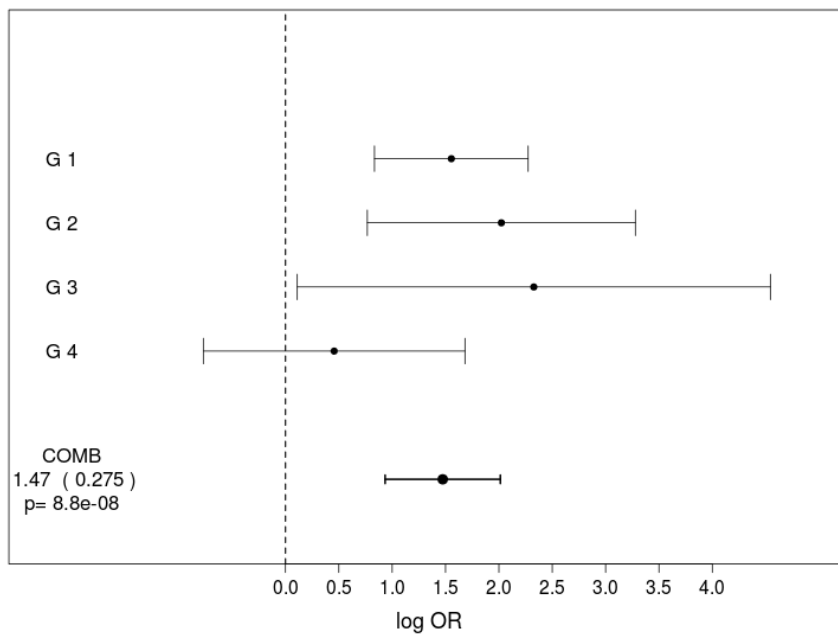
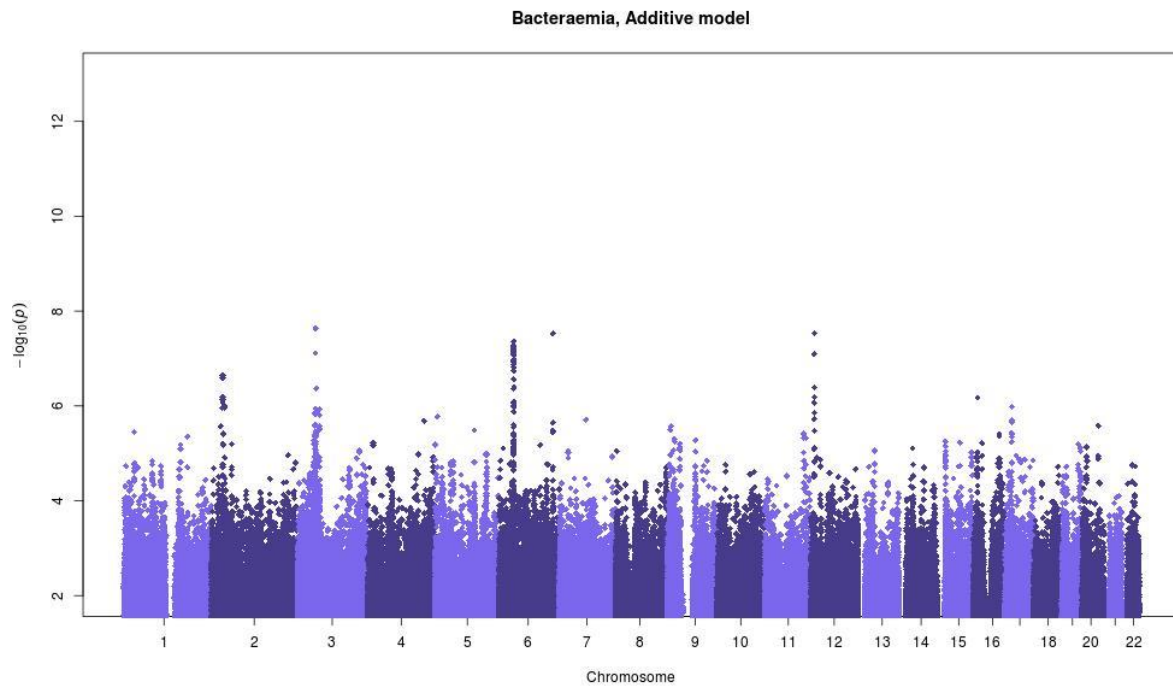


Figure S12. Manhattan plots for the bacteremia overall discovery analysis after imputation in the additive (A) and the genotypic (B) models. SNPs with minor allele frequency of > 2% and a model specific info value > 0.8 are included (10,996,498 and 8,156,976 SNPs in the additive and genotypic models respectively).

A)



B)

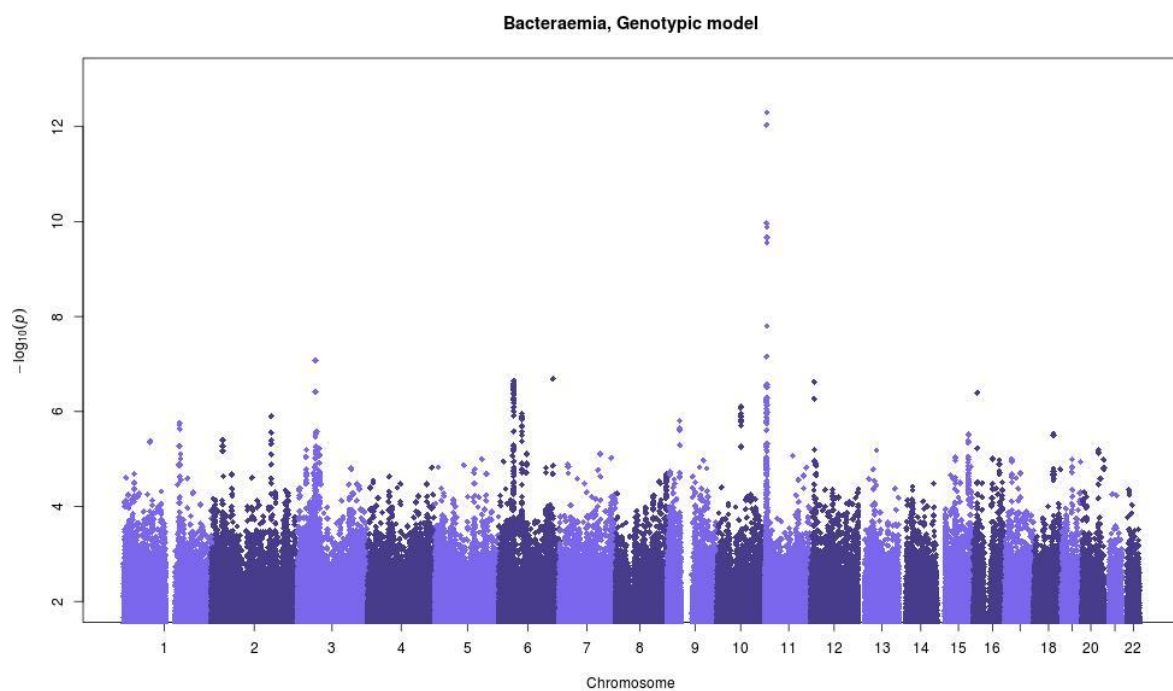
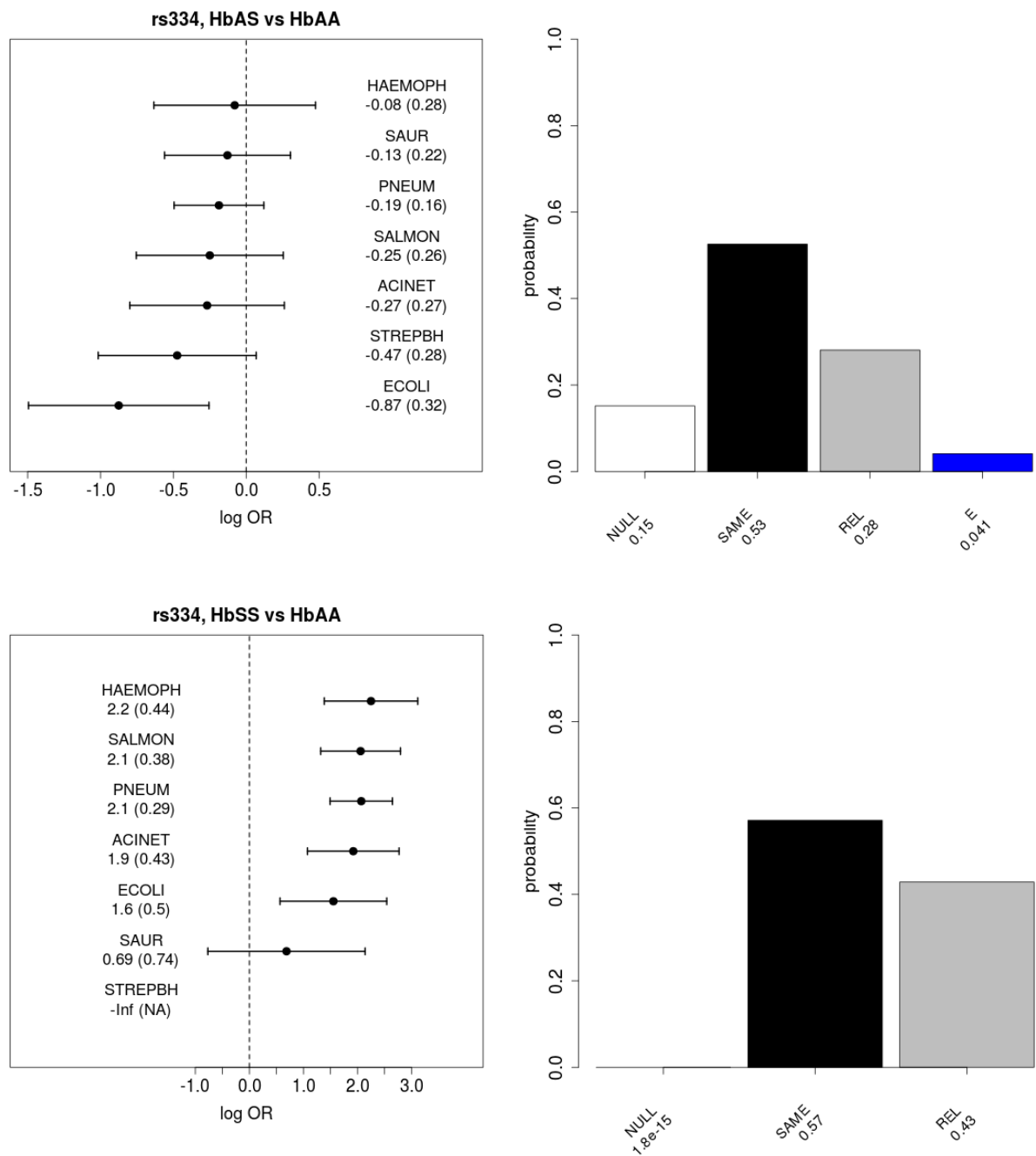


Figure S13. *HBB* association (rs334) with the main bacterial infections. Left panel: Log transformed combined odds ratios and 95% confidence intervals of directly genotyped discovery and replication samples. The dotted line represents the log OR of 0 (OR of 1; no difference between cases and controls). The values of point estimates and standard errors (in parentheses) are also given. Bacterial infections: PNEUM=*Streptococcus pneumoniae* (pneumococcus); ACINET=*Acinetobacter* species; HAEMOPH= *Haemophilus influenzae*; ECOLI=*Escherichia coli*; SALMON= *Salmonella* (non-typhoidal); STREPBH=*Streptococcus beta hemolytic*; SAUR=*Staphylococcus aureus*. Right panel: The posterior probabilities on the models of association: no effect in any subtype (NULL), same effect in all subtypes (SAME), related effects across subtypes (REL) or a same non-zero effect only in ECOLI (E) (See Methods). Models are a priori assumed to be equally likely. Bayes factors, which compare the evidence (marginal likelihood) between any pair of models, can be calculated as the ratio of the posterior probability assigned to each model as reported under each bar of the plot.



Supplemental Tables

Table S1. Sample exclusions in the discovery data and ImmunoChip replication sets.

Exclusion criteria	Number of excluded discovery samples	Number of excluded ImmunoChip replication samples
Call rate and extreme heterozygosity	182	84
HapMap PCA outliers	186	51
Outlying channel intensity	14	4
Sequenom discordance (N that otherwise passed the QC)	17 (5)	8 (7)
Discrepant or undetermined gender (N that otherwise passed the QC)	110 (32)	5 (0)
Intentional duplicate samples for QC purposes	16	0
Duplicate samples	87	6
Relatedness ($r > 0.4$)	117	29
Duplicates when compared with discovery samples	NA	29
Exclusions based on phenotype (altogether):	130	37
Missing clinical data	3	0
Contaminant, not bacteremia	9	0
Fungemia, not bacteremia	4	0
Meningitis, not bacteremia	44	0
Not bacteremia	25	1
Pneumonia, empyema, not bacteremia	15	0
Uncertain pathogen	30	1
Malaria, not bacteremia	0	35
Samples after QC	4213	1770

Table S2. Demographic data for cases and controls in discovery and replication sets.

	Cases (discovery; N=1536)	Cases (replication; N=434)	Controls (discovery; N=2677)	Controls (replication; N=1336)
Mean age in years (range)*	2.3 (0-13)	2.1 (0-13)	5.1 (0-6.9)	4.9 (0-6.7)
Proportion of individuals aged <2 months	16.5 %	26.5 %	0.19%	0.2%
Proportion of individuals aged 2 months – 2 years	47.4 %	39.7 %	5.0%	6.4%
Proportion of individuals aged 2 – 5 years	21.2 %	22.5 %	23.8%	43.8%
Proportion of individuals more than 5 years	14.8 %	11.4 %	70.7%	49.3%
Missing age information	0	0	0.3%	0.3%
Bacteremia	100%	100%	0.3%	0.2%
Mortality	25.8 %	26.1 %	0.9%	1.9%
Females	42.7 %	47.0 %	49.6 %	49.5 %
Reported ethnicity: Giriama	57.8 %	54.3 %	45.8 %	40.0 %
Reported ethnicity: Chonyi	24.9 %	25.0 %	37.1 %	44.1 %
Reported ethnicity: Kauma	7.3 %	12.5 %	11.9 %	10.3 %

*Controls belong to a birth cohort, hence all these children were less than 12 months of age when recruited to the study. The age of controls listed in this table refers to the age at the latest follow-up date.

Table S3. Distribution of the most common bacterial species in the discovery (1536 cases) and replication sets (434 cases).

Bacterial species	Discovery samples	Replication samples
<i>Streptococcus pneumoniae</i>	429 (27.9%)	113 (26.0%)
<i>Salmonella</i> (non-Typhi)	180 (11.7%)	38 (8.8%)
<i>Staphylococcus aureus</i>	178 (11.6%)	46 (10.6%)
<i>Escherichia coli</i>	162 (10.6%)	43 (9.9%)
<i>Streptococcus beta-hemolytic</i>	160 (10.4%)	48 (11.1%)
<i>Haemophilus influenzae</i>	134 (8.7%)	31 (7.1%)
<i>Acinetobacter</i> species	130 (8.5%)	48 (11.1%)

Table S4. Association results of the bacteremia overall and pneumococcal bacteremia analyses (additive test) after direct genotyping. GWAS discovery analysis (logistic regression using the first two principal components as covariates in PLINK) included 1536 cases with microbiologically proven bacteremia (429 pneumococcal) and 2677 controls. Approximately 2000 SNPs were included in the ImmunoChip replication (approximately $P < 1 \times 10^{-3}$), which were genotyped in 434 cases (113 pneumococcal) and 1336 controls. All of the SNPs with $P < 1 \times 10^{-5}$ in the discovery analyses are included in the table. In addition, SNPs with $P < 10^{-3}$ are shown, if they replicated in the same direction with $P < 0.05$. SNPs highlighted in bold show suggestive association and replication in the same direction, although none of these reached the genome-wide significance level of $P < 5 \times 10^{-8}$. OR = odds ratio; “Effects” shows the direction of association in the discovery and replication sets (“+” = minor allele confers a risk; “-” = minor allele is protective).

CHR	SNP	BP (b37)	Sub phenotype	P discovery	OR discovery	P replication	OR replication	P combined	OR combined	Effects	Gene ID
1	rs6669008	114166561	overall	0.000884	1.27	0.00078	1.48	4.71E-06	1.32	++	MAG13
2	rs207413	31539280	overall	1.32E-06	1.58	0.9735	0.99	1.76E-05	1.44	+-	
2	rs6716368	139924118	overall	0.000127	1.26	0.02504	1.27	9.24E-06	1.26	++	
3	rs10510428	14617925	overall	0.000253	1.18	0.02187	1.2	1.57E-05	1.19	++	
3	rs3922843	38624343	overall	0.000113	0.81	0.04668	0.83	1.47E-05	0.82	--	SCN5A
3	rs9869826	50998816	overall	2.88E-08	0.71	0.7553	1.03	4.43E-06	0.79	-+	DOCK3
3	rs7633054	62138954	overall	8.23E-07	1.39	0.04803	1.27	1.45E-07	1.36	++	PTPRG
3	rs9853409	62157762	overall	3.67E-06	1.34	0.3831	1.10	8.40E-06	1.28	++	PTPRG
4	rs4694744	70326656	overall	2.98E-05	0.65	0.00635	0.57	7.22E-07	0.63	--	
4	rs1080755	70344641	overall	0.000203	0.68	0.0265	0.65	1.57E-05	0.68	--	UGT2B4
4	rs13116232	70358525	overall	0.000219	0.68	0.0145	0.60	1.12E-05	0.67	--	UGT2B4
4	rs1500801	169668377	overall	0.000885	1.52	0.00068	1.99	3.96E-06	1.64	++	PALLD
5	rs11959975	9902829	overall	0.000229	1.25	0.0067	1.33	5.32E-06	1.27	++	LOC285692
5	rs7700462	74332599	overall	0.000224	1.26	0.0234	1.29	1.55E-05	1.27	++	GCNT4
5	rs7725038	145388180	overall	9.91E-06	1.34	0.1132	1.20	3.98E-06	1.3	++	SH3RF2
5	rs9325001	145393315	overall	9.00E-06	1.34	0.2409	1.14	1.06E-05	1.29	++	SH3RF2
6	rs12181583	42154609	overall	6.10E-06	0.8	0.5767	0.95	2.79E-05	0.83	--	
6	rs9369346	42159132	overall	1.13E-07	0.75	0.2948	1.10	5.67E-05	0.83	-+	<i>GUCA1B</i>
6	rs13217993	42164401	overall	5.33E-06	0.76	0.2594	1.12	0.000858	0.84	-+	<i>GUCA1B</i>
6	rs6912658	42167161	overall	4.39E-07	0.77	NA	NA	NA	NA	NA	<i>GUCA1B</i>
6	rs4424082	42194235	overall	3.32E-07	0.76	0.01507	1.26	0.001433	0.86	-+	<i>MRPS10</i>
6	rs1871693	136475578	overall	0.000313	0.79	0.04618	0.80	3.85E-05	0.79	--	PDE7B
6	rs4286796	152681049	overall	3.44E-06	1.30	0.9791	1.00	5.43E-05	1.22	+-	<i>SYNE1</i>
7	rs6961481	25256245	overall	9.84E-06	0.79	0.1371	1.14	0.002531	0.87	-+	<i>NPVF</i>
11	rs800160	2380134	overall	7.90E-05	1.23	0.00695	1.27	1.91E-06	1.24	++	
11	rs10840270	9629553	overall	3.34E-05	0.80	0.02075	0.80	2.04E-06	0.8	--	
11	rs17185574	61745694	overall	9.34E-06	0.39	0.3221	1.34	0.002646	0.59	-+	
12	rs9652020	40547379	overall	0.000212	0.80	0.01168	0.77	7.90E-06	0.79	--	
12	rs2873193	133484722	overall	0.000478	1.21	0.03069	1.23	3.79E-05	1.22	++	CHFR
17	rs11655073	31736656	overall	6.53E-06	2.02	NA	NA	NA	NA	NA	<i>ACCN1</i>
18	rs2111287	54933562	overall	0.000626	0.86	0.04462	1.18	7.23E-05	0.85	--	

18	rs7240462	69662581	overall	4.35E-05	0.83	0.06037	0.86	7.51E-06	0.84	--	
1	rs6669271	156738623	pneumococcal	1.05E-06	1.50	0.6652	0.93	2.73E-05	1.37	+-	<i>OR6Y1</i>
1	rs11264997	156752106	pneumococcal	3.81E-06	1.47	0.7004	0.94	7.57E-05	1.34	+-	
1	rs12145401	156761317	pneumococcal	6.03E-06	1.46	0.9775	1.00	5.08E-05	1.35	+-	
1	rs2054991	156772707	pneumococcal	5.06E-06	1.46	0.6475	0.93	0.000102	1.34	+-	
1	rs10908677	156772810	pneumococcal	2.30E-06	1.46	0.6475	0.93	0.000049	1.34	+-	
1	rs923663	156774356	pneumococcal	8.37E-06	1.45	0.6475	0.93	0.00015	1.33	+-	
1	rs863345	156801606	pneumococcal	6.17E-07	1.63	NA	NA	NA	NA	NA	
3	rs3913941	21468206	pneumococcal	0.00036	1.39	0.03406	1.45	0.000036	1.4	++	<i>ZNF659</i>
3	rs4858325	21468776	pneumococcal	0.00057	1.37	0.04191	1.43	6.44E-05	1.39	++	<i>ZNF659</i>
4	rs7661138	156124175	pneumococcal	3.30E-06	1.55	0.01081	0.54	0.000661	1.35	+-	
5	rs6452345	26604000	pneumococcal	6.73E-07	3.70	0.3556	0.39	4.69E-06	3.22	+-	
6	rs1570187	123982387	pneumococcal	8.22E-06	0.71	0.4033	0.89	1.59E-05	0.75	--	<i>TRDN</i>
7	rs2723392	13395217	pneumococcal	0.00094	1.40	0.01648	1.57	5.14E-05	1.44	++	
7	rs1347077	13396229	pneumococcal	0.00093	1.40	0.01648	1.57	5.14E-05	1.44	++	
7	rs17155006	107545079	pneumococcal	0.00017	0.75	0.01443	0.70	7.90E-06	0.74	--	<i>LAMB4</i>
8	rs3739262	134274895	pneumococcal	2.51E-06	1.63	0.4737	1.17	5.23E-06	1.53	++	<i>WISP1</i>
13	rs9526114	45306038	pneumococcal	4.93E-05	1.35	0.04376	1.33	5.83E-06	1.35	++	<i>LOC283514</i>
14	rs10483548	38386980	pneumococcal	2.27E-06	1.45	0.5388	0.91	7.56E-05	1.32	+-	

Table S5. The most significant associations (additive model) in the pneumococcal bacteremia discovery analysis after imputation. Imputed discovery analysis (logistic regression using the first two principal components as covariates in SNPTEST) included 429 cases with microbiologically proven pneumococcal bacteremia and 2677 controls. All the loci with $P < 1 \times 10^{-6}$ in SNPTEST analysis are included in the table (Only the most significant SNP in each locus plus the ones with replication information available). In addition, SNPs with $P < 1 \times 10^{-5}$ are shown, if replication genotyping was available. SNPs highlighted in bold show suggestive association and replication. Replication genotyping was performed using the Sequenom iPLEX platform (103 cases and 1333 controls), unless denoted with a * in which case the ImmunoChip was used (113 cases and 1336 controls). OR = odds ratio; “Effects” shows the direction of association in the discovery and replication sets (“+” = minor allele confers a risk; “-” = minor allele is protective).

Directly genotyped	CHR	BP (b37)	SNP	GENE	P discovery imputed	OR discovery imputed	MAF imputed cases	MAF imputed controls	MAF genotyped cases discovery	MAF genotyped controls discovery	P genotyped discovery	OR genotyped discovery	P Replication	OR Replication	P Combined	OR Combined	Effects
---	1	62932815	rs1168000	DOCK7	3.81E-06	2.53	0.0396	0.0183	0.0287	0.0112	3.686E-05	2.81	0.04553	3.10	4.64E-06	2.85	++
---	1	62953183	rs1168025	DOCK7	3.98E-07	3.07	0.0329	0.0123	0.0276	0.0112	9.211E-05	2.70	0.05296	2.97	1.32E-05	2.74	++
---	1	158464990	rs11264984	-	3.80E-06	1.41	0.5072	0.4253	0.5266	0.4405	2.851E-06	1.42	0.8489	0.97	4.55E-05	1.31	+-
YES	1	158471999	rs6669271	-	8.37E-07	1.50	0.2867	0.2092					*0.6652	*0.93	2.73E-05	1.37	+-
---	1	158473725	rs6663286	-	2.15E-06	1.45	0.3439	0.2654	0.3338	0.2525	1.996E-06	1.49	0.3933	0.87	9.55E-05	1.34	+-
YES	1	158485482	rs11264997	-	3.29E-06	1.47	0.2949	0.2219					*0.7004	*0.94	7.57E-05	1.34	+-
YES	1	158494693	rs12145401	-	5.17E-06	1.45	0.2937	0.2223					*0.9775	*1.00	5.08E-05	1.35	+-
YES	1	158506083	rs2054991	-	6.30E-06	1.45	0.2937	0.2228					*0.6475	*0.93	0.000102	1.34	+-
YES	1	158506186	rs10908677	-	2.47E-06	1.46	0.3019	0.2260					*0.6475	*0.93	0.000049	1.34	+-
YES	1	158507732	rs923663	OR6Y1	6.30E-06	1.45	0.2937	0.2228					*0.6475	*0.93	0.00015	1.33	+-
YES	1	158518939	rs9804152	OR6Y1	9.00E-06	1.57	0.1539	0.0996					*0.6851	*1.09	4.48E-05	1.45	++
---	1	158528118	rs10752626	OR6P1	3.11E-07	1.64	0.1906	0.1231	0.1916	0.1216	2.725E-07	1.65	0.6004	1.11	1.17E-06	1.54	++
YES	1	158534982	rs863345	OR6P1	2.10E-07	1.65	0.1946	0.1253	0.1937	0.1241	3.766E-07	1.65	0.506	1.15	1.14E-06	1.55	++
---	3	128701130	rs6439153	KIAA1257	4.71E-07	3.72	0.0251	0.0077	0.0413	0.0225	0.0001585	2.18	0.000608	3.02	4.99E-07	2.39	++
YES	4	155904725	rs7661138	-	3.02E-06	1.54	0.2133	0.1526					*0.01081	*0.54	0.000661	1.35	+-
YES	5	26568243	rs6452345	-	8.26E-08	3.70	0.0280	0.0075					*0.3556	*0.39	4.69E-06	3.22	+-
---	5	169453092	rs114183953	DOCK2	1.95E-07	3.15	0.0335	0.0111	0.0337	0.0108	8.934E-07	3.19	0.562	1.36	2.34E-06	2.77	++
---	5	169460440	rs138715413	DOCK2	1.81E-07	3.16	0.0334	0.0109	0.0323	0.0110	3.778E-06	3.00	0.5461	1.38	8.06E-06	2.63	++
YES	6	38520864	rs724242	BTBD9	8.76E-06	0.64	0.1539	0.2185					*0.91	*0.98	0.000121	0.72	--
YES	6	123940688	rs1570187	TRDN	7.49E-06	0.71	0.4114	0.4836					*0.4033	*0.89	1.59E-05	0.75	--
---	7	13394635	rs188755755	-	8.91E-09	2.72	0.0593	0.0220	0.0544	0.0214	1.039E-06	2.50	0.01096	2.40	3.73E-08	2.47	++
---	7	13396630	rs140817150	-	7.25E-09	2.74	0.0592	0.0217	0.0639	0.0266	3.585E-07	2.39	0.001161	2.72	1.69E-09	2.47	++
---	7	13401014	rs116432683	-	7.20E-09	2.74	0.0592	0.0217									
---	8	134193074	rs11782936	-	5.80E-07	1.65	0.2035	0.1404									
YES	8	134205713	rs3739262	WISP1	1.98E-06	1.63	0.1667	0.1087					*0.4737	*1.17	5.23E-06	1.53	++
YES	9	27773059	rs775397	-	8.98E-06	1.46	0.2622	0.1946					*0.8725	*1.03	0.000057	1.36	++
---	14	39314125	rs7142347	-	3.69E-07	1.74	0.1475	0.0963									
---	14	39315768	rs73277180	-	4.71E-07	1.73	0.1470	0.0961	0.1396	0.0966	6.451E-05	1.56	0.9459	1.02	0.000224	1.46	++
YES	14	39317229	rs10483548	-	1.96E-06	1.45	0.3835	0.3106					*0.5388	*0.91	7.56E-05	1.32	+-

Table S6. The most significant associations in the chr7 lincRNA region in the pneumococcal bacteremia discovery analysis (after Affymetrix SNP 6.0 genotyping and imputation). Directly genotyped SNPs with P-values less than 0.05 and imputed SNPs with P-value less than 1×10^{-6} are shown in the region (250 kb region flanking the top SNP rs140817150 is shown). * denotes imputed SNPs that were confirmed by direct genotyping using Sequenom.

SNP	BP (b37) in chr 7	genotyped(G)/imputed (I)	P discovery	OR discovery	maf cases	maf controls	Position relative to AC011288.2-001	Position relative to AC006000.5
rs6973224	13266540	G	0.043	1.17	0.371	0.340	intron 2-3	3' downstream
rs17166908	13270959	G	0.0037	0.78	0.223	0.273	intron 2-3	3' downstream
rs10230302	13275542	G	0.0016	1.33	0.220	0.170	intron 2-3	3' downstream
rs183536610	13350258	I	2.05E-07	2.42	0.061	0.027	intron 3-4	3' downstream
rs114563489	13351620	I	1.76E-07	2.44	0.061	0.026	intron 3-4	3' downstream
rs114237419	13352333	I	1.62E-07	2.44	0.061	0.026	intron 3-4	3' downstream
rs184608123	13355876	I	1.15E-07	2.48	0.061	0.026	intron 3-4	3' downstream
rs112384903	13356316	I	1.12E-07	2.48	0.061	0.026	intron 3-4	3' downstream
rs75811201	13360996	I	9.13E-08	2.50	0.061	0.025	intron 3-4	3' downstream
rs75208382	13363234	I	1.42E-07	2.51	0.059	0.024	intron 3-4	3' downstream
rs192359699	13365219	I	5.45E-08	2.55	0.061	0.025	intron 3-4	3' downstream
rs184746152	13371007	I	4.31E-08	2.57	0.060	0.024	intron 3-4	3' downstream
rs75091393	13373716	I	4.14E-08	2.58	0.060	0.024	intron 3-4	3' downstream
rs139819665	13374686	I	4.11E-08	2.58	0.060	0.024	intron 3-4	3' downstream
rs186811037	13377949	I	4.10E-08	2.58	0.060	0.024	intron 3-4	3' downstream
rs182261263	13378675	I	4.08E-08	2.58	0.060	0.024	intron 3-4	3' downstream
rs146813781	13380872	I	4.07E-08	2.58	0.060	0.024	intron 3-4	intron 3-4
rs182683147	13385227	I	4.04E-08	2.58	0.060	0.024	intron 3-4	intron 3-4
rs74757989	13386451	I	4.04E-08	2.58	0.060	0.024	intron 3-4	intron 3-4
rs78571337	13387037	I	4.03E-08	2.58	0.060	0.024	intron 3-4	intron 3-4
rs186096483	13387766	I	4.01E-08	2.58	0.060	0.024	intron 3-4	intron 3-4
rs116118621	13391291	I	1.08E-08	2.70	0.059	0.022	intron 3-4	intron 3-4
rs188755755*	13394635	I	8.91E-09	2.72	0.059	0.022	intron 3-4	intron 3-4
rs140817150*	13396630	I	7.25E-09	2.74	0.059	0.022	intron 3-4	intron 3-4
rs116432683	13401014	I	7.20E-09	2.74	0.059	0.022	intron 3-4	intron 3-4
rs114325568	13423766	I	1.43E-07	2.44	0.062	0.026	intron 3-4	5' upstream
rs2568600	13424671	G	0.0012	1.39	0.171	0.131	intron 3-4	5' upstream
rs2723392	13428692	G	0.00097	1.40	0.175	0.133	intron 3-4	5' upstream
rs1347077	13429704	G	0.00097	1.40	0.175	0.133	intron 3-4	5' upstream
rs77922834	13432361	I	1.06E-08	2.70	0.061	0.023	intron 3-4	5' upstream
rs192669340	13471405	I	2.66E-08	2.49	0.068	0.029	intron 3-4	5' upstream
rs184390046	13476586	I	1.79E-08	2.70	0.058	0.023	intron 3-4	5' upstream
rs10807764	13478678	G	0.0061	1.28	0.223	0.177	intron 3-4	5' upstream
rs17167126	13480033	G	0.00014	1.43	0.193	0.139	intron 3-4	5' upstream
rs2217612	13483120	G	0.0029	1.35	0.172	0.129	intron 3-4	5' upstream
rs17167140	13484133	G	0.001827	1.38	0.171	0.127	intron 3-4	5' upstream
rs17167142	13484328	G	0.001568	1.38	0.171	0.127	intron 3-4	5' upstream
rs1432488	13484508	G	0.00209	1.37	0.171	0.128	intron 3-4	5' upstream
rs10247084	13485416	G	0.003145	1.33	0.185	0.143	intron 3-4	5' upstream
rs1432492	13501571	G	0.015065	1.23	0.256	0.213	intron 3-4	5' upstream
rs12699509	13561595	G	0.013637	1.34	0.114	0.090	intron 3-4	5' upstream

Table S7. Comparison of association statistics after imputation and direct genotyping of discovery and replication samples. As described in the Methods section, only siblings (and more related individuals) were excluded from the main analysis. Association statistics of the following analyses are shown: the imputed discovery main analysis (SNPTEST2; frequentist method score, the first two principal components as covariates), directly genotyped discovery and replication analyses (PLINK, 2 PCA components as covariates), directly genotyped discovery and replication analyses when a mixed model (MM) was used to better account for relatedness and underlying population structure (with siblings and more distant relatives included and with all second degree and more closely related individuals ($r>0.2$) excluded from the analysis). Number of bacteremia cases overall (cases B), pneumococcal cases (cases P), and controls included in each analysis are indicated in each column. The chromosome 7 lincRNA hit in the pneumococcal subgroup (SNPs rs188755755 and rs140817150) reached genome-wide significance in the additive model in all of these analyses. MM =mixed model; B = bacteremia overall; P = pneumococcal bacteremia.

CHR	BP (b37)	SNP	Phenotype	Discovery (Imputed)		Discovery (Genotyped)		Replication (Genotyped)		Combined (Genotyped)		MM Discovery (Genotyped; siblings included)		MM Replication (Genotyped; siblings included)		MM Combined (Genotyped; siblings included)		MM Discovery (Genotyped; all related ($r>0.2$) removed)		MM Replication (Genotyped; all related ($r>0.2$) removed)		MM Combined (Genotyped; all related ($r>0.2$) removed)	
				cases B: 1536 cases P: 429 controls: 2677	OR	cases B: 1514 cases P: 418 controls: 2642	OR	cases B: 407 cases P: 103 controls: 1333	OR	cases B: 1921 cases P: 521 controls: 3975	OR	cases B: 1519 cases P: 420 controls: 2688	OR	cases B: 408 cases P: 103 controls:1360	OR	cases B: 1927 cases P: 523 controls: 4048	OR	cases B: 1476 cases P: 408 controls:2543	OR	cases B: 404 cases P: 102 controls:131 1	OR	cases B: 1880 cases P: 510 controls: 3854	OR
				P	OR	P	OR	P	OR	P	OR	P	OR	P	OR	P	OR	P	OR	P	OR	P	OR
3	51009435	rs9879725	B	2.4E-08	0.71	6.9E-08	0.71	0.5085	1.07	1.6E-05	0.79	8.0E-08	0.72	0.3019	1.11	4.0E-05	0.80	0.02839	0.80	0.947	1.01	0.0564	0.84
3	51390967	rs13353505	B	3.8E-06	0.64	3.2E-05	0.67	0.9783	1.00	0.0004	0.75	8.4E-05	0.70	0.8907	1.02	0.00095	0.77	0.00832	0.66	0.891	1.04	0.0272	0.74
6	42162388	rs3749921	B	7.9E-08	0.74	2.3E-07	0.75	0.1653	1.14	0.0002	0.83	4.1E-07	0.76	0.1525	1.14	0.00028	0.84	0.01349	0.80	0.414	1.15	0.0771	0.87
6	42181960	rs1884318	B	4.4E-08	0.74	3.9E-07	0.75	0.1632	1.14	0.0002	0.84	7.2E-07	0.76	0.1501	1.14	0.0004	0.85	0.0155	0.80	0.412	1.15	0.0848	0.87
6	42184400	rs13197564	B	5.3E-08	0.74	1.6E-07	0.75	0.1493	1.15	0.0002	0.83	3.2E-07	0.76	0.1416	1.14	0.00025	0.84	0.01139	0.80	0.474	1.13	0.0601	0.86
6	152678110	rs6906442	B	3.6E-06	1.35	3.2E-05	1.32	0.536	1.08	8.1E-05	1.26	5.8E-05	1.30	0.6408	1.06	0.00017	1.24	0.06383	1.22	0.738	0.93	0.1268	1.16
6	152679729	rs9478326	B	2.3E-06	1.30	1.9E-05	1.27	0.8464	1.02	0.0001	1.20	6.3E-05	1.24	0.9014	1.01	0.00038	1.18	0.04085	1.20	0.587	0.91	0.1122	1.14
12	9350854	rs74500107	B	8.1E-08	1.80	3.3E-07	1.68	0.3738	0.85	4.7E-05	1.44	8.0E-07	1.63	0.3668	0.84	7.9E-05	1.41	0.0763	1.36	0.685	0.87	0.1621	1.24
12	9358781	rs149632973	B	4.1E-07	1.75	2.8E-06	1.70	0.3227	0.82	0.0003	1.43	9.9E-06	1.63	0.3286	0.82	0.0006	1.39	0.14336	1.33	0.883	0.95	0.2233	1.23
1	62932815	rs1168000	P	3.8E-06	2.53	3.7E-05	2.81	0.0455	3.10	4.6E-06	2.85	3.0E-05	2.76	0.0463	2.90	4.6E-06	2.79	8.5E-05	2.65	0.041	2.99	1.2E-05	2.70
1	62953183	rs1168025	P	4E-07	3.07	9.2E-05	2.70	0.053	2.97	1.3E-05	2.74	8.2E-05	2.64	0.0541	2.78	1.4E-05	2.66	0.00022	2.53	0.049	2.85	3.4E-05	2.58
1	158464990	rs11264984	P	3.8E-06	1.41	2.9E-06	1.42	0.8489	0.97	4.6E-05	1.31	7.2E-07	1.45	0.8720	0.98	2.1E-05	1.33	1.5E-06	1.44	0.868	0.98	3.8E-05	1.32
1	158473725	rs6663286	P	2.2E-06	1.45	2E-06	1.49	0.3933	0.87	9.6E-05	1.34	1.9E-06	1.47	0.4061	0.87	0.00011	1.33	6.8E-07	1.50	0.433	0.88	5.4E-05	1.35
1	158528118	rs10752626	P	3.1E-07	1.64	2.7E-07	1.65	0.6004	1.11	1.2E-06	1.54	7.6E-08	1.68	0.6203	1.11	6.2E-07	1.55	2.8E-07	1.66	0.627	1.10	1.9E-06	1.53
1	158534982	rs863345	P	2.1E-07	1.65	3.8E-07	1.65	0.506	1.15	1.1E-06	1.55	1.1E-07	1.68	0.5136	1.14	5.8E-07	1.56	3.7E-07	1.65	0.520	1.14	1.7E-06	1.54
5	169453092	rs114183953	P	1.9E-07	3.15	8.9E-07	3.19	0.562	1.36	2.3E-06	2.77	1.7E-07	3.39	0.6620	1.24	1.4E-06	2.82	4.1E-07	3.34	0.592	1.31	2.3E-06	2.79
5	169460440	rs138715413	P	1.8E-07	3.16	3.8E-06	3.00	0.5461	1.38	8.1E-06	2.63	1.0E-06	3.16	0.6367	1.27	5.2E-06	2.67	2.2E-06	3.11	0.569	1.33	8.6E-06	2.64
7	13394635	rs188755755	P	8.9E-09	2.72	1E-06	2.50	0.011	2.40	3.7E-08	2.47	2.6E-07	2.57	0.0060	2.59	9.2E-09	2.58	1.7E-07	2.65	0.004	2.72	4.6E-09	2.67
7	13396630	rs140817150	P	7.3E-09	2.74	3.6E-07	2.39	0.0012	2.72	1.7E-09	2.47	9.7E-08	2.45	0.0005	2.96	5.1E-10	2.55	3.6E-08	2.56	3E-4	3.06	1.5E-10	2.66
14	39315768	rs73277180	P	4.7E-07	1.73	6.5E-05	1.56	0.9459	1.02	0.0002	1.46	9.7E-05	1.54	0.9666	0.99	0.00044	1.43	0.0001	1.54	0.962	0.99	0.0005	1.43
16	26762549	rs142637346	P	1.28E-06	2.36	3.7E-06	2.37	0.5861	1.25	9.2E-06	2.13	1.8E-06	2.38	0.5383	1.29	5.3E-06	2.15	2.7E-06	2.37	0.563	1.27	8.2E-06	2.13
16	26775675	rs140127109	P	1.07E-06	2.37	4.2E-06	2.36	0.6124	1.23	1.1E-05	2.11	2.0E-06	2.37	0.5656	1.27	6.1E-06	2.14	2.8E-06	2.37	0.591	1.25	9.1E-06	2.12
16	26786471	rs148926738	P	1.11E-06	2.35	8.9E-05	2.16	0.3186	1.47	8.1E-05	2.00	6.1E-05	2.17	0.2866	1.50	5.7E-05	2.01	0.0001	2.14	0.306	1.48	9.7E-05	1.98

Table S8. The most significant associations (additive and genotypic models) in the bacteremia overall discovery analysis after imputation. Imputed discovery analysis (logistic regression using the first two principal components as covariates in SNPTTEST) included 1536 cases with microbiologically proven bacteremia and 2677 controls. All the loci with $p < 1 \times 10^{-6}$ in the SNPTTEST analysis are included in the table (Only the most significant SNP in the locus plus the ones with replication information available are shown). In addition, SNPs with $< 1 \times 10^{-5}$ are shown, if replication genotyping was available. SNPs highlighted in bold show suggestive association and replication. Replication genotyping was performed using the Sequenom iPLEX platform (407 cases and 1333 controls), unless denoted with * in which case the ImmunoChip was used (434 cases and 1336 controls). Only the additive model is shown if both of the models passed the criteria described above. OR = odds ratio; “Effects” shows the direction of association in the discovery and replication sets (“+” = minor allele confers a risk; “-” = minor allele is protective).

Directly genotyped	CHR	BP (b37)	SNP	GENE	P discovery imputed	OR discovery imputed	MAF imputed cases	MAF imputed controls	MAF genotyped cases	MAF genotyped controls	P genotyped discovery	OR genotyped discovery	P Replication	OR Replication	P Combined	OR Combined	Effects	Model
YES	1	30169447	rs11808436		3.58E-06	1.25	0.359	0.309					*0.2624	*0.91	0.0005	1.16	+-	Additive
---	2	31300106	rs17010685	<i>GALNT14</i>	2.27E-07	2.18	0.031	0.017	0.043	0.035	0.04829	1.26	0.1479	0.70	0.25224	1.13	+-	Additive
YES	2	31539280	rs207413		1.09E-06	1.58	0.076	0.051					*0.9735	*0.99	1.8E-05	1.44	+-	Additive
YES	3	50998816	rs9869826	<i>DOCK3</i>	2.30E-08	0.71	0.150	0.202					*0.7553	*1.03	4.4E-06	0.79	+-	Additive
---	3	51009435	rs9879725	<i>DOCK3</i>	2.36E-08	0.71	0.151	0.203	0.147	0.196	6.9E-08	0.71	0.5085	1.07	1.6E-05	0.79	+-	Additive
---	3	51390967	rs13353505	<i>DOCK3</i>	3.84E-06	0.64	0.054	0.080	0.057	0.082	3.2E-05	0.67	0.9783	1.00	0.00037	0.75	+-	Additive
---	3	53783784	rs898420	<i>CACNA1D</i>	4.25E-07	1.28	0.384	0.342										Additive
YES	3	62138954	rs7633054	<i>PTPRG</i>	1.18E-06	1.38	0.151	0.110					*0.04803	*1.27	1.5E-07	1.36	++	Additive
YES	3	62157762	rs9853409	<i>PTPRG</i>	2.95E-06	1.34	0.174	0.135					*0.3831	*1.10	8.4E-06	1.28	++	Additive
---	3	62160653	rs17066238	<i>PTPRG</i>	2.96E-06	1.45	0.109	0.078	0.116	0.091	0.00022	1.33	0.8184	0.97	0.00186	1.23	+-	Additive
YES	6	42154609	rs12181583	<i>GUCA1B</i>	5.89E-06	1.25	0.253	0.301					*0.5767	*0.95	2.8E-05	0.83	--	Additive
YES	6	42159132	rs9369346	<i>GUCA1B</i>	1.18E-07	0.75	0.208	0.264					*0.2948	*1.10	5.7E-05	0.83	+-	Additive
---	6	42162388	rs3749921	<i>GUCA1B</i>	7.90E-08	0.74	0.201	0.257	0.202	0.257	2.3E-07	0.75	0.1653	1.14	0.00017	0.83	+-	Additive
YES	6	42164401	rs13217993	<i>GUCA1B</i>	3.92E-06	0.76	0.169	0.217					*0.2594	*1.12	0.00086	0.84	+-	Additive
---	6	42181960	rs1884318	<i>MRPS10</i>	4.35E-08	0.74	0.200	0.257	0.202	0.257	3.9E-07	0.75	0.1632	1.14	0.00024	0.84	+-	Additive
---	6	42184400	rs13197564	<i>MRPS10</i>	5.29E-08	0.74	0.207	0.264	0.208	0.264	1.6E-07	0.75	0.1493	1.15	0.00015	0.83	+-	Additive
YES	6	42194235	rs4424082	<i>MRPS10</i>	2.74E-07	0.76	0.205	0.261					*0.01507	*1.26	0.00143	0.86	+-	Additive
---	6	152672540	rs4632900	<i>SYNE1</i>	2.96E-08	1.41	0.188	0.145										Additive
---	6	152678110	rs6906442	<i>SYNE1</i>	3.57E-06	1.35	0.163	0.129	0.148	0.117	3.2E-05	1.32	0.536	1.08	8.1E-05	1.26	++	Additive
---	6	152679729	rs9478326	<i>SYNE1</i>	2.25E-06	1.30	0.232	0.189	0.239	0.199	1.9E-05	1.27	0.8464	1.02	0.00013	1.20	++	Additive
YES	6	152681049	rs4286796	<i>SYNE1</i>	3.30E-06	1.30	0.221	0.179					*0.9791	*1.00	5.4E-05	1.22	+-	Additive
---	12	9322394	rs75698727	<i>PZP</i>	2.92E-08	1.78	0.066	0.038										Additive
---	12	9350854	rs74500107	<i>PZP</i>	8.06E-08	1.80	0.060	0.035	0.067	0.039	3.3E-07	1.68	0.3738	0.85	4.7E-05	1.44	+-	Additive
---	12	9358781	rs149632973	<i>PZP</i>	4.10E-07	1.75	0.057	0.034	0.055	0.033	2.8E-06	1.70	0.3227	0.82	0.00028	1.43	+-	Additive
---	16	12323566	rs830715	<i>SNX29</i>	6.75E-07	1.29	0.300	0.251										Additive
---	10	67559700	rs16921870		7.94E-07	NA	0.094	0.079										Genotypic
YES	10	67560490	rs10509235		8.23E-07	NA	0.095	0.079					*0.0255					Genotypic
YES	11	5029457	rs11035102	<i>HBB</i>	6.93E-08	NA	0.245	0.245					*0.2618					Genotypic
---	11	5273865	rs113892119	<i>HBB</i>	5.08E-13	NA	0.096	0.093	0.083	0.085	3.42E-12	NA	0.00178	NA	NA	NA		Genotypic
---	11	5252794	rs12295158	<i>HBB</i>	1.32E-10	NA	0.124	0.132	0.123	0.129	2.89E-08	NA	0.2385	NA	NA	NA		Genotypic
---	11	5248232	rs334	<i>HBB</i>	1.33E-10	NA	0.136	0.129	0.087	0.085	4.09E-11	NA	0.00077	NA	NA	NA		Genotypic